

SAFETY OF 12-MONTH CREATINE SUPPLEMENTATION COMBINED

WITH RESISTANCE TRAINING IN OLDER ADULTS

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Graduate Studies and Research

In Partial Fulfillment of the Requirements

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University of Saskatchewan

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By

TIMOTHY A. LANDERYOU

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ABSTRACT

Introduction: Creatine is a nitrogen-containing compound that is found in many supplements that claim to increase muscle mass and there is increasing evidence that creatine supplementation can increase muscle mass in older individuals.

Purpose: To evaluate the safety of 12-months creatine supplementation and resistance exercise in older adults by monitoring measures of kidney and liver function, complete blood count, and reports of adverse events.

Methods: Older adults (n=70, males 50 years of age or older (39), and post-menopausal females (31)) who were not performing resistance exercise were recruited for the study and randomized by computer to creatine (32; 18 males, 14 females) or placebo (38; 21 males, 17 females) groups. Other exclusionary criteria were: presence/history of kidney impairments, consumption of creatine supplement/bone altering drugs, and the presence of fragility fractures. Participants were given creatine or placebo (0.1g/kg/day) consumed before and after resistance training on exercise days and with a meal on non-exercise days. The study used a double-blind, placebo-controlled design. Blood and urine were collected to assess complete blood cell count, liver function indicators (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and Bilirubin), and creatinine clearance (kidney function) at baseline, 4 months, 8 months, and 12 months. A mixed ANOVA was used to determine differences in the kidney and liver measures within and between groups, and chi-square analysis was used to determine if the frequency counts for liver, kidney, and other adverse events were different

between groups.

Results: There were no significant differences found in either the mixed ANOVA or chi-square analysis for liver and kidney function measures. There was a significant difference ($p=0.033$) found between groups in the chi-square analysis for gastrointestinal (GI)/Cramping Adverse Events (16 Creatine vs. 6 Placebo).

Conclusion: The present study indicates that creatine supplementation of 0.1g/kg/day in older adults for a 12-month period has no adverse effects on liver or kidney function but can cause cramping or GI distress in some individuals.

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Last I would like to acknowledge my parents, whose support and character provided me the passion and dedication to complete this thesis, whose gift of curiosity has guided me through my academic career, and whose encouragement and love have paved the road for me all my life.

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DEDICATION

This thesis is dedicated to my wife Kaitlyn, who at certain points over the course of this project, seemed to want it completed even more than I did!

Our adventure began shortly after the beginnings of this thesis, so it seems fitting that it be dedicated to her. Through dating, a yearlong engagement, and even a marriage, this project has been a part of our lives, and even though some of these (as well as other) events made giving time to this thesis difficult, in the end it's hard to have regrets after some of the great times we've had.

Kaitlyn came along at the perfect time in my life to not only provide love and support for me through this project, but in all other areas as well. I can't wait to start the next chapter of an already fantastic adventure with her.

I love you with all my heart Kaitlyn, and this thesis is dedicated to you.

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LIST OF ABBREVIATIONS

AE – Adverse Event

AGAT – Glycine Amidinotransferase

ALP – Alkaline Phosphatase

ALT – Alanine Transaminase

ANOVA – Analysis of Variance

AST – Aspartate Aminotransferase

ATP – Adenine Triphosphate

CEP – Certified Exercise Physiologist

Cr - Creatine

CrCl – Creatinine Clearance

CSEP – Canadian Society for Exercise Professionals

ECCLS – European Committee on Clinical Laboratory Standards

GAA – Guanidino Acetic Acid

GAMT – Guanidinoacetate Methyltransferase

GFR – Glomerular Filtration Rate

GI - Gastrointestinal

HTIB – [Hydroxy(tosyloxy)iodo]benzene

ICH – International Conference on Harmonisation

IFCC – International Federation of Clinical Chemistry

KDOQI – Kidney Disease Outcomes Quality Initiative

MAT - Adenosyltransferase arginine

NAD – Nicotinamide Adenine Dinucleotide (oxidized)

NADH – Nicotinamide Adenine Dinucleotide (reduced)

NKF – National Kidney Foundation PCr - Phosphocreatine

RM – Repetition Maximum

SCr – Serum Creatinine

SD – Standard Deviation

UCr – Urine Creatinine

V – Volume

1. INTRODUCTION

1.1 Creatine

Creatine is a nitrogen containing compound that is found in high concentrations in type II muscle fibers. Approximately 95% of the body's creatine stores are located in skeletal muscle with the remaining 5% found in the brain, liver, kidneys, and testes (Persky & Brazeau, 2001).

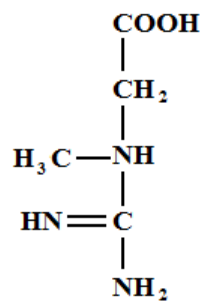


Figure 1-1. Molecular Structure of Creatine

Creatine is contained in many supplements that claim to increase muscle mass and since its introduction in the early 90s, has widely increased in its popularity and use. Sales of creatine containing supplements were estimated to be \$14 million in the US in 2011 (Eudy, 2013). While the supplement is primarily used by young adults and athletes, there is increasing evidence that creatine supplementation can increase muscle mass in older individuals. (Brose et al, 2003, Candow et al., 2008; Chrusch et al., 2001; Gotshalk et al., 2002 and 2008; Rawson et al., 1999; Harris et al., 2011).

1.2 Ergogenic Action

Creatine acts by increasing phosphocreatine (PCr) stores within skeletal muscle (Vandenberghe et al., 1997 and 1999). PCr stores are responsible for high-energy, forceful contractions from skeletal muscle by allowing quick resynthesis of ATP. They also aid in regeneration of ATP stores in “microdomains” which are vital in importance for maintaining cardiac/skeletal muscle and brain function (Figure 1.2). These “microdomains”, or cellular compartments, are located in the sarcoplasmic reticulum membrane and the myofibrils near the sarcolemma of cardiac and skeletal muscle cells and the cellular membrane of brain cells (Guzan et al., 2011). At these sites, phosphate is cleaved from PCr by Creatine Kinase and combined with ADP to produce ATP.

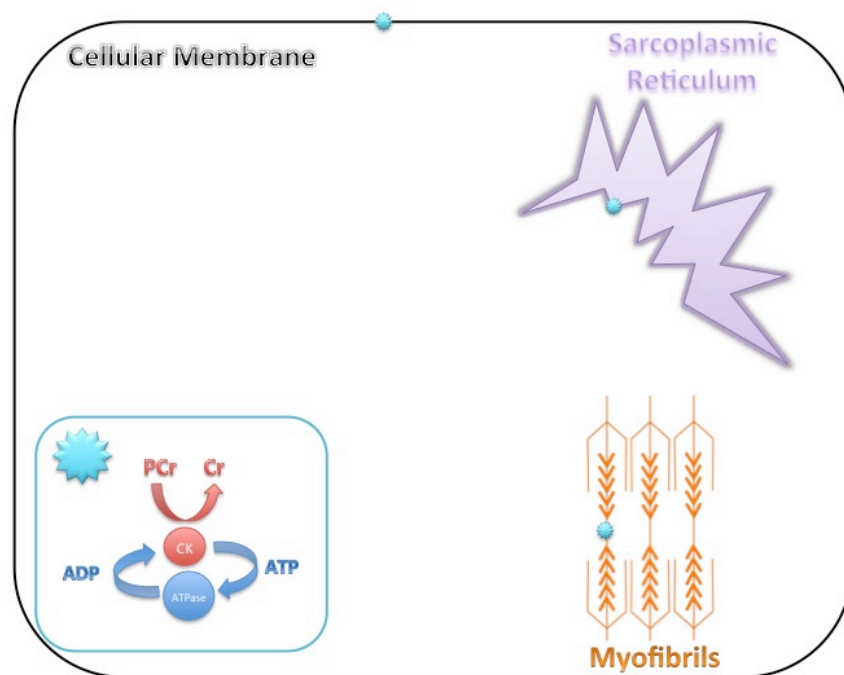


Figure 1-2. Illustration of ATP synthesis in microdomains. Adapted from Guzan et al., 2011.

1.3 Creatine Metabolism

While the amount of creatine stored by the body varies greatly, by sex, age, and muscle mass, it has been estimated that a 70 kg male will store approximately 120g of total creatine (Brosnan, et al., 2011). Stored creatine is either synthesized using the amino acids glycine, methionine, and arginine, or absorbed through direct dietary ingestion of creatine. The most abundant sources of dietary creatine are animal proteins, specifically red meat and fish (such as salmon and tuna).

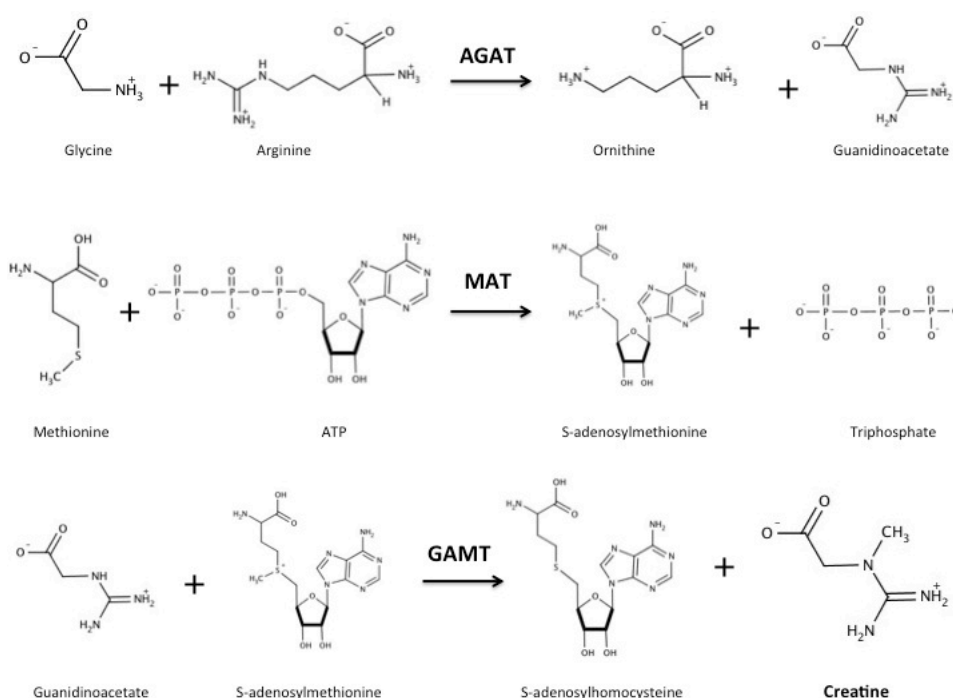


Figure 1-3. Creatine Synthesis from Amino Acids. Adapted from Brownan et al., 2011.

Figure 1-3 (above) outlines the chemical reactions involved in creatine synthesis from amino acids. Creatine synthesis requires three enzymes: methionine adenosyltransferase (MAT), arginine:glycine amidinotransferase (AGAT), and guanidinoacetate methyltransferase (GAMT)

and is an inter-organ process. Guanidino acetic acid (GAA) is synthesized in the kidneys by incorporating one molecule of glycine and one amidino group from arginine. It is then transported to the liver where a methyl group from methionine is added along with an ATP to produce creatine and A-adenosylhomocysteine (Brosnan et al., 2011).

Once in the blood stream, creatine follows one of two pathways, as outlined in Figure 1-4.

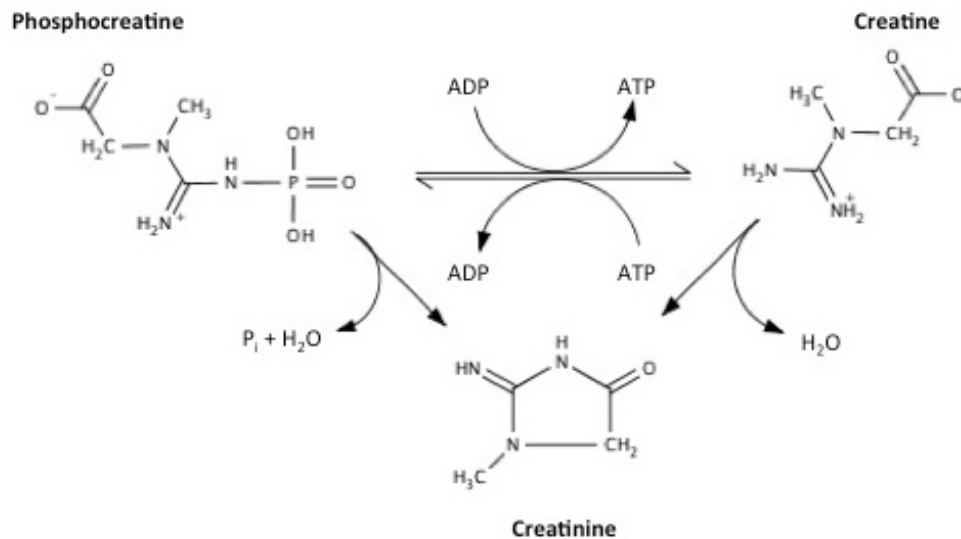


Figure 1-4. Pathways of Creatine Metabolism Following Oral Ingestion. Adapted from Persky & Brazeau, 2001.

The first is conversion to PCr to saturate cells or tissues within the body (as described above) and the second is renal elimination. Approximately 90-95% of creatine in the body is stored in skeletal muscle as either free (Cr) or phosphorylated (PCr) creatine. Once the body's storage domains are saturated, any remaining creatine is converted to creatinine and is excreted by the

kidneys. (Persky & Brazeau, 2001). Creatinine is also produced during skeletal muscle contractions as a byproduct of PCr metabolism (Fig. 1-4) to produce ATP and thus creatinine levels are largely dependent on individual muscle mass.

Factors such as age, lean body mass, and amount of ingested creatine have a large impact on levels of excreted creatinine. Estimates for the conversion of creatine to creatinine in healthy individuals are 1.6-1.7% of the total creatine pool per day (Brosnan et al., 2011) and the normal range for 24-hour urine creatinine collection is 500-2000 mg (Creatinine – urine, 2013).

1.4 Creatine and Aging

A progressive decline in muscle mass and strength occurs as a result of aging (Rawson & Venezia, 2011; Candow & Chilibeck, 2005; Evans, 1995). One possible cause for this decline is a reduction in high-energy phosphate metabolism. Most PCr (90-95%) is stored in skeletal muscle (Smith et al., 1998) and therefore a loss of skeletal muscle mass with age would arguably cause a reduction in PCr (Moller et al., 1980). The positive effects for creatine on increasing muscle mass and strength have been well documented by the literature in both animal (Ferreira et al, 2005) and young adult populations (Persky & Brazeau, 2001; Rosene et al., 2009) and there is increasing evidence for positive effects of creatine supplementation in the aging population as well (Brose et al, 2003; Candow et al., 2008; Chrusch et al., 2001; Gotshalk et al., 2002 and 2008; Rawson et al., 1999, 2002, and 2004; Canete et al., 2006; Harris, 2011). Other studies have found no effects of creatine supplementation in older adults (Eijnde et. al, 2003; Bermon et. al, 1998). A possible explanation for why some studies of older adults supplementing with creatine did not find significant results could be the nature of Cr storage. PCr is stored primarily in

skeletal muscle and is much more prevalent in Type II vs. Type I muscle fibres. Older adults could see an attenuated effect of creatine supplementation on muscle mass and performance for two reasons: Muscle mass decreases with age and there is a shift from Type II to Type I fibres, with preferential atrophy of Type II fibres (Deschenes, 2004). This theory is supported by research that shows muscle PCr increases significantly more in young adults supplementing with creatine versus older adults (Rawson et al., 2002). Other researchers have found that base levels of PCr are heavily influential on gains associated with Cr supplementation and in some cases older adults can see increases greater than younger adults (REF). Further research of the effects of creatine supplementation on older adults is required.

1.5 Safety & Side Effects

1.5.1 Animal Studies

While there is no conclusive evidence of negative side effects of creatine supplementation, there have been inconsistent reports that point to the need for caution when using creatine, especially within special populations or if using high doses (Kim et al., 2011). Since creatine is synthesized in the liver and creatinine is excreted by the kidneys, creatine supplementation in populations who may already have pre-existing conditions which compromise kidney or liver function (liver/kidney disease) would be contraindicated. Studies performed on animals supplementing with high doses (1-5g/kg/day) of creatine showed increased liver enzyme, urea, and creatinine levels in the blood, as well as structural changes to both the liver and kidneys, which indicate tissue damage (Souza et al., 2009). Tarnopolsky et al. (2003) showed mice, but not rats, developed hepatic inflammatory lesions when supplementing with creatine monohydrate while another study found creatine supplementation increased cyst growth and worsened renal function

in rats (Edmunds et al., 2001). The authors pointed out differences in metabolism of creatine based on species and urged future studies to consider these differences when evaluating the physiological and toxicological implications of supplementing with creatine.

1.5.2 Human Studies

In humans, two individuals with abnormal kidney function (interstitial nephritis and focal glomerular sclerosis) saw improvement upon termination of creatine supplementation (Koshy et al., 1999; Pritchard & Kalra, 1998). Both these studies have been refuted by other authors (Tarnopolsky, 2010), who attribute the impaired renal function in these individuals to other medications (cyclosporine and non-steroidal inflammatory drugs) they were taking at the time.

While the above cases are serious, they are isolated and many other studies have shown no adverse effects of creatine supplementation on the liver or kidneys (Gualano et al., 2008; Tarnopolsky, 2010). The absence of liver or kidney dysfunction has also been observed in studies supplementing with high doses of creatine (30g/day) and over long periods of time (up to 5 years) (Poortmans et al., 1997; Poortmans & Francaux, 1999).

Following those studies, Yu & Deng (2000) showed a metabolic pathway by which creatine is converted to methylamine and then formaldehyde. This pathway is outlined in Figure 1-5, found on the following page. More recent research (Sale et. al., 2009) also showed increased methylamine concentration in individuals supplementing with creatine at a dose of 4 x 5g/day. Methylamine, which is deaminated to formaldehyde in this manner, has been linked to vascular damage, complications with diabetes, and nephropathy.

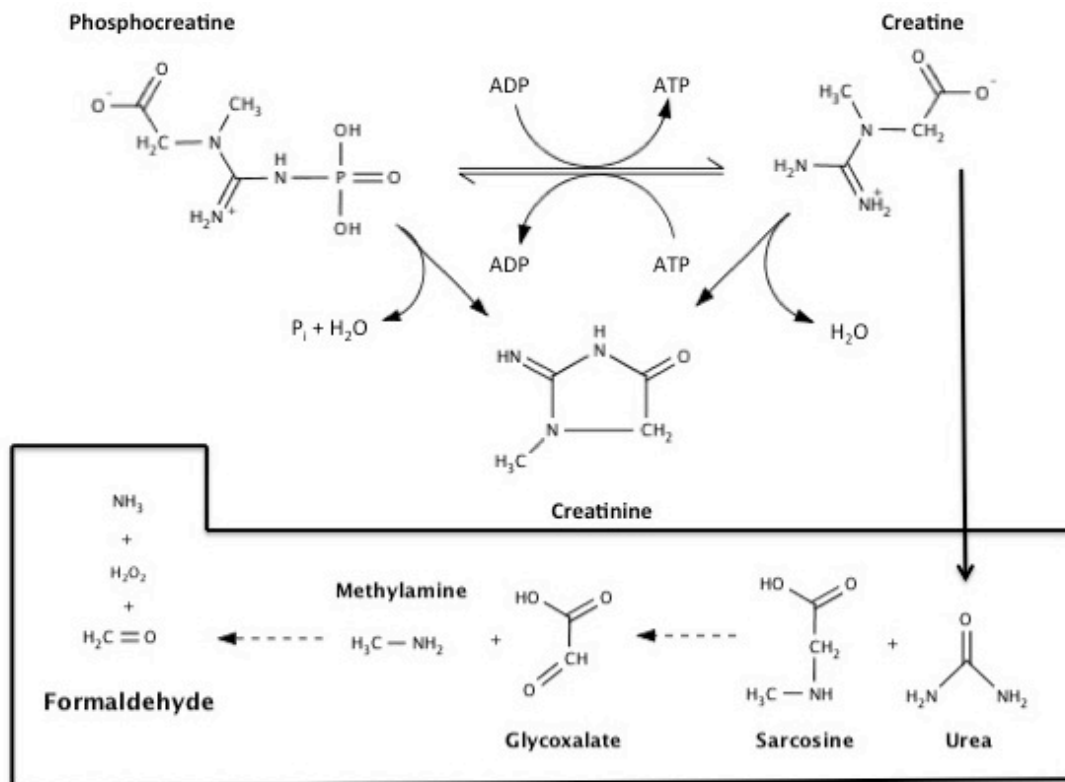


Figure 1-5. Metabolic Conversion Pathway of Creatine to Formaldehyde. Adapted from Persky & Brazeau, 2001.

Concern about this pathway may be premature however, as a more recent study found no difference in formaldehyde excretion between older individuals supplementing with creatine versus placebo (Candow et al., 2008). This study did use a low dose of creatine (0.1g/kg), which could have prevented the build-up of formaldehyde. Another study showed an increased excretion of methylamine and formaldehyde in younger men while supplementing with creatine, but that it had no effect on the normal function of the kidneys (Poortmans et al., 2005). Some researchers (Sale et al., 2009) have also shown that changing the supplement delivery at a given

amount can significantly reduce the formation of methylamine with creatine supplementation. In their study, methylamine excretion was significantly reduced at a dosage of 20x1g/day versus 4 x 5g/day. This would indicate that for most individuals, creatine supplementation can be safe but to use caution when working with certain populations with compromised kidney function, or at risk of kidney disease, such as older adults.

Claims of other side effects as a result of creatine supplementation have also been cited. These include: gastrointestinal (GI) discomfort, diarrhea/loose stool, dehydration and cramping. GI symptoms have been cited in several studies with incidence rates as high as 35% (Juhn, 1999, Chrusch, et al., 2001, Groenvelde, 2005). Other studies have found low or no incidence of these symptoms though, so it remains to be seen whether this is a function of creatine itself or simply an improperly administered supplement or dosage. One study which cited a 5% incidence rate attributed even this low number to the supplement being improperly self-administered and that the symptoms could have been eliminated by taking the supplement with meals and/or better mixing it into a viscous medium such as yogurt (Tarnopolsky, 2010). In most cases, GI related side effects are also participant reported, and thus will suffer from large variations in reported incidence as a result. A more standardized approach to reporting side effects of this nature is clearly needed for accurate and meaningful comparisons across studies.

Other side effects such as muscle cramping and muscle pulls/strains have been reported in the literature (Chrusch et al., 2001) as well. These side effects were originally thought to occur due to dehydration (Williams & Branch, 1998), which creatine supplementation could cause by increasing intracellular water retention (Dalbo et al., 2008). Volek et al. (2001) found that while

creatine supplementation increases the aldosterone response, it did not significantly change temperature, blood pressure, sweat rate, or sodium/potassium excretion. More recent studies have also shown creatine supplementation to have no significant effect on several different hydration and thermoregulatory variables (Lopez, 2009), so if creatine supplementation increases risk of cramping or muscle pulls/strains, dehydration is not likely the mechanism responsible.

Another theory that could explain an increased incidence of cramping or muscle pulls/strains with creatine supplementation is a higher training volume/intensity. Optimizing creatine stores in skeletal muscle could allow participants to push harder in training and cramp as a result. This theory is supported by research, which showed increased training volume (kg x reps) in the creatine group versus placebo (Chrusch et al. 2001; Wiroth et al., 2001; Candow et al., 2008). The reporting of all these side effects has been inconsistent, or anecdotal, and in most cases not significant in the literature and thus more evidence is needed to confirm or refute these claims (Persky & Brazeau, 2001; Kim et al., 2011).

1.5.3 Older Adults

It is evident that the safety and efficacy of creatine use in young, healthy populations is not especially controversial, as many studies have been done both in the long and short term with minimal and/or anecdotal claims of adverse side effects (Kim et al., 2011). As the therapeutic effects of creatine ingestion are further explored, they will continue to be used on other populations (such as the elderly) and on a more chronic/long-term basis, thus, the safety of such use needs to be evaluated more thoroughly. Several studies have been done to assess possible positive effects (Brose et al, 2003; Candow et al., 2008; Chrusch et al., 2001; Gotshalk et al.,

2002 and 2008; Rawson et al., 1999, 2002, and 2004; Canete et al., 2006; Harris, 2011) of creatine in an older population. Various research groups have reported benefits of creatine supplementation in older adults, observing everything from increases in aerobic and anaerobic exercise performance (Wiroth et al., 2001), functional movement tasks (Canete et al., 2006), and body composition (Rawson et al., 2004). Few of these studies took safety and/or side effect measurements (Chrusch et al., 2001; Brose et al., 2003), and they were all short in length (<3 months). Chrusch et al. (2001) found significantly greater incidences of loose stool, muscle cramping, and muscle pull/strain in the creatine vs. placebo groups, while Brose et al. (2003) found no significant difference in side effects between groups. Neves et al. (2011) also found no difference in the glomerular filtration rate (GFR – a measure of kidney function) of post-menopausal women taking creatine vs. placebo for 12 weeks.

At present there have been very few studies done to assess the safety of creatine ingestion in older adults over a long period of time. Groenveld et al. (2005) found no significant differences between creatine and placebo groups for renal function measurements, or incidences of nausea, GI discomfort, and diarrhea over a period of 10 months in patients with ALS. Three participants, all in the creatine group, had to stop intake of creatine during the course of the study due to severe diarrhea (2) and severe nausea (1). Bender et al. (2008) observed markers of renal function and reports of GI disturbances in older adults with Parkinson's Disease taking either creatine or placebo over a period of two years. Serum creatinine was significantly different between groups at 12 months, but was not significantly different before or after that. This indicates possible renal involvement in creatine metabolism, but with no progressive decline in kidney health (which likely would have been indicated by steadily higher serum creatinine

levels) it does not appear to adversely affect kidney function. Researchers did see significantly higher reporting of GI related complaints after 24 months. None of these studies used creatinine clearance (widely regarded as the gold standard for kidney function) as an outcome measure, but relied on other measures to estimate GFR.

1.5.4 Kidney Function

Creatinine clearance (CrCl) is a measure of the amount of creatinine excreted per unit time (usually measured in ml/min) and is used to estimate glomerular filtration rate (GFR) thereby giving an indication of kidney function. This test requires levels of serum and urine creatinine (SCr and UCr) to be measured along with 24-hour urine volume (V) in order to estimate GFR using the following equation:

$$\text{CrCl} = (\text{UCr} \times \text{V}) / \text{SCr}$$

Creatinine clearance is directly correlated with muscle mass and so it is affected by age, sex, and body composition as a result. Females, older adults, and smaller individuals will have lower CrCl than males, younger adults, and larger individuals, so CrCl can vary widely by individual. The U.S. National Library of Medicine online lists the normal range for CrCl in healthy adults as 97-137ml/min for males and 88-128ml/min for females (A.D.A.M., 2013). Due to variance in age, gender, and laboratory procedures, normal ranges for creatinine clearance will also vary by facility. The present study used a normal range of 74-125 ml/min (C.C.P.E., 2013). Creatinine clearance levels that exceed the normal range usually indicate an increased ingestion of creatine, either from diets high in meat, or supplementation, as creatinine is released into the blood as a by-product of creatine metabolism. Elevated creatinine clearance may also be seen during and

after exercise, due to muscle damage that occurs which can leak creatine or creatinine into the blood (Machado et al., 2012). High creatinine clearance is rarely observed, and is not usually a concern, especially in individuals supplementing with creatine, because it indicates an increased level of urine creatinine, which simply means that the body is excreting additional creatinine.

Creatinine clearance below the normal range however, is a concern, because creatinine is passively filtered by the kidneys. According to the American Association for Clinical Chemistry (A.A.C.C.), any disease or condition that affects the glomeruli can decrease the kidneys' ability to clear creatinine and other wastes out of the blood. When this occurs, serum creatinine will be increased and creatinine clearance will be decreased because not enough creatinine is being filtered out through the kidneys and excreted in the urine (A.A.C.C., 2012).

A decreased creatinine clearance rate may also occur when there is decreased blood flow to the kidneys as may occur with congestive heart failure, a kidney obstruction, or acute or chronic kidney failure. The less effective kidney filtration is, the less creatinine can be filtered through them and excreted, and the greater the decrease in creatinine clearance will be (A.A.C.C., 2012).

1.5.5 Liver Function

Liver function is primarily assessed by measuring the levels of bilirubin and liver enzymes – alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST), in the blood. Low levels of these substances in the blood are not normally cause for concern. Elevated levels can indicate various health concerns depending on which substance is elevated, the level of elevation, and the ratio of these substances to each other.

Increased ALT in the blood can indicate acute ($>10\times$ normal range) or chronic ($<4\times$ normal range) hepatitis, and mild increases can also be seen in bile duct obstructions, cirrhosis, heart damage, alcohol abuse, and hepatic cancer/tumors (A.A.C.C., 2013).

AST is elevated in similar circumstances to ALT. This includes acute ($>10\times$ normal range) and chronic ($<4\times$ normal range) hepatitis, bile duct obstructions, cirrhosis, and certain liver cancers. AST will also be elevated after heart attacks and muscle injury, usually much higher (3-5x) than ALT, and will stay elevated for longer than ALT with liver injury. For these reasons, ALT and AST are often compared to each other to determine the nature of the illness. (A.A.C.C., 2013).

Increased ALP levels in the blood will also occur in instances of hepatitis, bile duct obstructions, and liver cancer. In hepatitis, ALP is much less elevated than AST and ALT, while if the bile ducts are blocked, ALP will be increased (along with bilirubin) much more than AST and ALT. Elevated ALP can also indicate increased bone cell activity, which could occur with bone diseases such as Paget's or cancers that have spread to the bone. Moderately increased ALP can also be caused by Hodgkin's lymphoma, congestive heart failure, ulcerative colitis, and certain bacterial infections. For these reasons, it is very important that ALP results are compared with other markers of liver function to determine whether the cause is liver related or indicative of some other disease. An ALP isoenzyme test can also be performed to determine if the high ALP is from bone or the liver, if the signs and symptoms or other routine tests are unclear (A.A.C.C., 2013).

Elevated bilirubin indicates liver dysfunction, though the nature is dependent on the type of bilirubin that is elevated. If unconjugated (indirect) bilirubin is high, it can indicate haemolytic or

pernicious anemia, cirrhosis, or a transfusion reaction. If conjugated (direct) bilirubin is high, it can indicate viral hepatitis, drug reactions, alcoholic liver disease, bile duct obstructions (due to gall stones or scarring), or liver tumors. If conjugated bilirubin levels reach high enough, bilirubin may also be present in the urine and is detectable with a dipstick test as part of a urinalysis (A.A.C.C., 2012).

1.6 Purpose

The present study aims to analyze the long-term safety of creatine use combined with resistance training in older adults by studying the effects on the liver, kidneys, and other side effects such as muscle cramping and GI discomfort, that are theorized to occur with creatine supplementation. This study aims to assess the safety of long-term creatine supplementation combined with resistance training within this population by answering the following questions:

- i) Does creatine supplementation combined with resistance training adversely affect liver or kidney function in older adults?
- ii) Does creatine supplementation combined with resistance training lead to increased incidence of GI symptoms (GI distress, diarrhea, dehydration) and cramping in older adults?

1.7 Hypothesis Statements

The following hypothesis statements have been created to scientifically answer the questions presented in the study purpose:

- i) Values for liver and kidney function indicators will not differ significantly between creatine and placebo groups.
- ii) The number of adverse events related to kidney and liver function will not differ significantly between creatine and placebo groups.
- iii) The number of adverse events related to GI symptoms (GI distress, diarrhea, dehydration) and cramping associated with creatine supplementation will not differ significantly between creatine and placebo groups.

2. METHODS

2.1 Study Design

The study used a double-blind, placebo-controlled, repeated measures design. Once recruited, participants were randomized using a computer-generated allocation schedule, performed by a research assistant independent from the rest of the study staff, to one of two groups: (1) resistance exercise and creatine supplementation or (2) resistance exercise and maltodextrin (placebo). This research assistant also prepared the study kits. Randomization was performed using a fixed block size of 4 and a permuted block design with a computer random number generator. This means participants were grouped into blocks of 4 and each block received an even distribution to creatine and placebo (2 each per block). This method was chosen to keep the intervention group sizes close to the same. The allocation sequence was concealed from the research assistant enrolling and assessing participants. All researchers who performed assessments, supervised training, and performed data entry, were blinded to group assignment.

Following randomization, there was a familiarization protocol for the resistance training exercises. The familiarization period lasted 2 weeks and the purpose was to reduce the amount of learning, which might contribute to strength increases in the initial stages of the exercise protocol. Following the familiarization period, baseline assessments were performed and participants were given their study kits. At 4 month intervals (4, 8, and 12 months) assessments were performed again to assess liver and kidney function. Adverse event reporting was undertaken throughout the study; participants were encouraged to report adverse events to a staff member, as well as proactively collected by staff members who supervised on training days. If

warranted, an Adverse Event Form (Appendix A) was completed and filed so that it could be included in further analysis. A diagram of the participant flow is seen below (Figure 2-1).

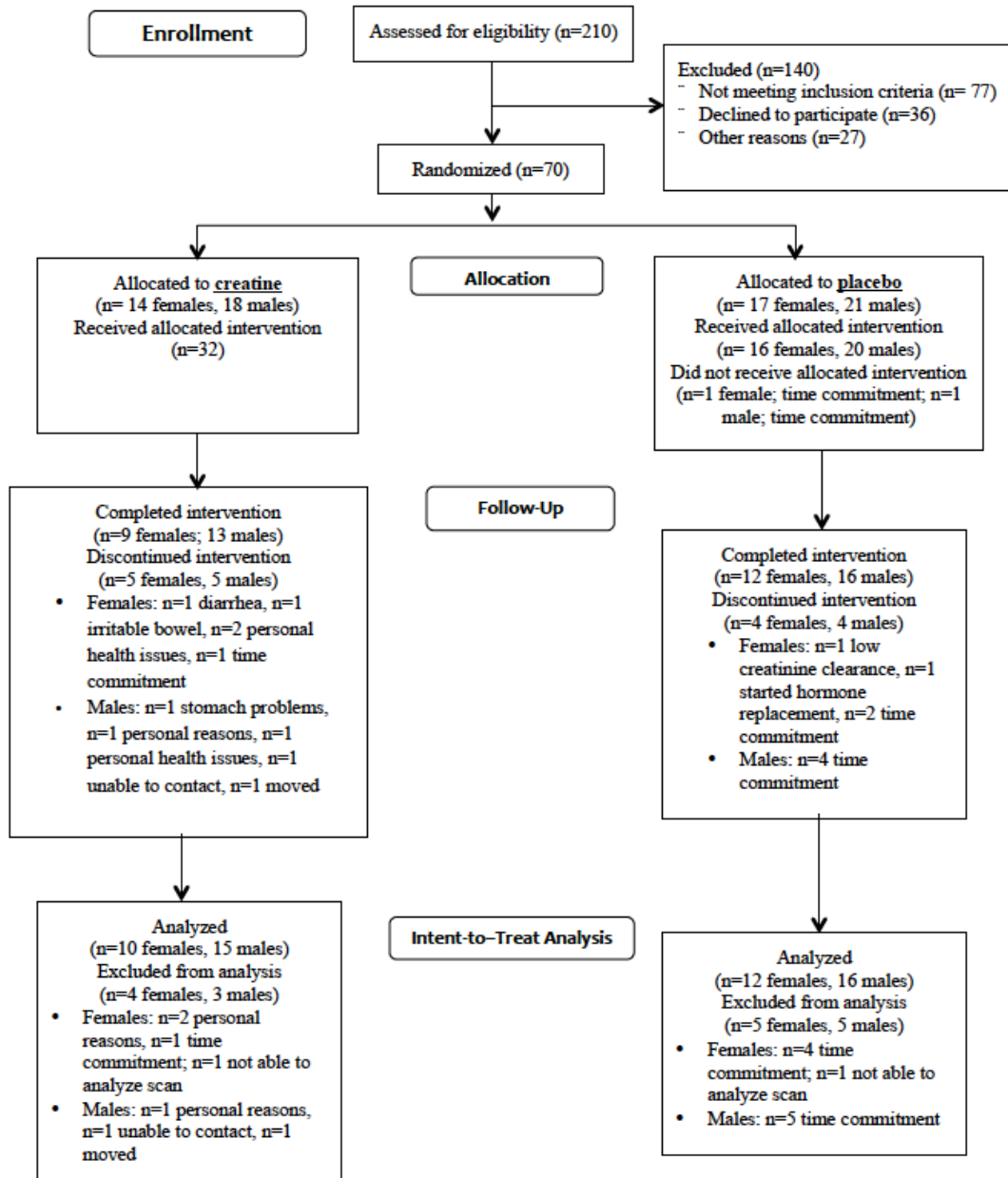


Figure 2-1. Participant Flow Chart

2.2 Participants

Table 2-1 (below) contains demographic data on the participants included in the study.

Table 2-1. Participant Demographic Data

Group	N	M/F	Mean Age	Mean Weight	Mean Height
Creatine	32	18/14	58.5	81.6	170.2
Placebo	38	21/17	57.6	81.2	169.8

Of the 210 individuals recruited for the present study, 140 did not meet inclusion criteria, 36 declined to participate, and 27 were excluded for other reasons. This left a sample of 70 individuals who participated. To be eligible for the study, individuals had to be men 50y and older, or post-menopausal women (no menstruation for at least 12 months). If women reported being post-menopausal for less than two years, menopausal status was verified by determining levels of follicle stimulating hormone and leutinizing hormone. Exclusion criteria were:

- pre-existing kidney abnormalities
- creatinine clearance values below the normal reference range
- previous fragility fractures
- history of taking medications that affect bone mineral density in the past year, including: bisphosphonates, parathyroid hormone, calcitonin, hormone replacement therapy, selective estrogen receptor modulators, parathyroid medication, or androgen therapy
- currently taking corticosteroids
- suffer from severe osteoarthritis
- have taken creatine supplementation within the past 6 months

- Crohn's disease, Cushing's disease, or severe osteoarthritis
- Currently involved in resistance training (more than 2x20minutes per week)

Participants were recruited via newspaper advertisements and posters circulated in the city of Saskatoon. Table 2-2 (below) indicates medications participants were taking at baseline.

Table 2-2. Participant Medications at Baseline

Group	Participant ID	Medication
Creatine	1	Meloxicam, nasonex
	6	Zocor, Celexa, aspirin
	8	Eltroxin, Citalopram
	32	Baby aspirin
Placebo	2	Calcium, multivitamin, glucosamine, Ginseng, Gingko biloba, Ibuprofen
	18	Crestor
	29	Micardis
	46	Fluoxetine, Nu-atenol, amlodipine, altace
	51	Synthroid, nortriptyline, Arthrotec, Aspirin
	61	Wellbutrin
	72	Hydrochlorothiazide, Teva-rabeprazole
	92	Citalopram
	211	Rosuvastatin, Cilazapril monohydrate

2.3 Exercise Protocol

Before performing the resistance exercise protocol, participants would warm-up on a stationary cycle ergometer for 5 minutes and perform light stretching. They would then perform 3 sets of 10 repetitions to muscular fatigue, with 2-minute rest sessions between sets for each exercise at an intensity corresponding to a load equal to or greater than 80% 1-RM (i.e. 80% of their maximum strength). Each resistance exercise session took approximately 60 minutes to complete

and was performed 3x/week under the supervision of a Certified Exercise Physiologist (CSEP-CEP) in a controlled environment.

Participants were encouraged to maintain a regular schedule; working out on the same days each week, with a 48-hour rest period between subsequent exercise days to minimize the risk of overtraining and fatigue.

The resistance exercises chosen for the protocol included: hack squat, chest press, lat pull down, shoulder press, leg curl/extension, biceps curl, triceps extension, calf press, and back extension.

These exercises were chosen as they trained all major muscle groups (Candow et al, 2006 and 2008; Chilibeck et al, 2005). Wrist flexion and extension and ankle plantar flexion/dorsiflexion were also used.

Participants maintained daily training logs where average training volume per session (weight x sets x repetitions) was determined and monitored for each participant by a supervisor. Resistance was individualized and increased by 1-5kg once the participant could complete 3 sets of 10 repetitions for that exercise.

Duration of the exercise protocol was 12 months and compliance with the exercise protocol was assessed through the use of logs.

2.4 Supplementation Protocol

Creatine monohydrate (RIVALUS Inc.) or placebo was orally administered with half before (~5mins) and half after (~5mins) completion of the resistance training protocol on exercise days and with meals on non-exercise days. Rationale for timing of supplementation and placebo was

that it is more effective for inducing muscle hypertrophy and increasing strength than at a time distant from training sessions (Cribb & Hayes, 2006). Both supplements were of the same dosage (0.1g/kg/day) and identical in appearance and taste. The placebo was isocaloric to creatine monohydrate and contained maltodextrin. Creatine monohydrate and placebo were administered in a double-blind fashion in the form of powder mixed with juice or milk. Dosage was chosen as it was shown to reduce bone resorption in older males (Candow et al., 2008). There are no “non-exercise” groups in the study design because creatine has minimal effects without exercise and its main ergogenic effect is to increase phosphocreatine stores to allow one to train with a higher exercise volume (Chrusch et al., 2001). The intervention duration was 12 months and compliance with the supplementation protocol was assessed through the use of logs. Participants were also surveyed after the study to assess the effectiveness of our blinding by asking if they thought they were on the creatine monohydrate, or the placebo, or were not sure.

2.5 Measures

The study relied on three types of measurements to confirm its hypotheses: blood and urine markers of liver and kidney function, liver and kidney adverse events, and other adverse events. Liver and kidney measurements were taken four times over the course of the study: baseline, 4 months, 8 months, and 12 months. Adverse events were recorded using adverse event forms throughout the study.

2.5.1 Liver Measurements

Serum bilirubin and three liver enzyme levels were measured as part of a standard blood panel, to assess liver health over the course of the study: alkaline phosphatase (ALP), alanine

aminotransferase (ALT), and aspartate aminotransferase (AST). All were measured using in vitro tests for their respective quantitative determinations in human serum and plasma on the Roche/Hitachi Cobas c501 module, which automatically calculates the analyte concentration of each sample. Reliability of testing equipment has been verified in previous research; the coefficients of variation (SD / mean) for the test analytes are less than 5% and meet working guidelines for analyte testing by the European Working Group, which has more demanding standards than the US CLIA (Smolcic et al., 2011). Data sheets containing additional information on these substances can be found as Appendix B. Reference ranges and analyte testing methods were obtained via information sheets from Cobas. These information sheets can be found as Appendix C.

2.5.2 Alkaline Phosphatase

ALP was assessed through colorimetric assay in accordance with methods standardized against the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) formulation (Tietz et al., 1983). In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic ALP activity and is determined by measuring the increase in absorbance.

2.5.3 Alanine Aminotransferase

ALT was assessed by assay, which follows the recommendations of the IFCC, but was optimized for performance and stability (Bergmeyer et al., 1985; ECCLS, 1989). ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. NADH reduces the pyruvate formed in a reaction catalyzed by lactate dehydrogenase to form L-lactate and NAD^+ .

2.5.4 Aspartate Aminotransferase

AST was assessed by assay that follows the recommendations of the IFCC, but was optimized for performance and stability (Bergmeyer et al., 1985, ECCLS, 1989). AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH in the presence of malate dehydrogenase to form NAD^+ . The rate of NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

2.5.5 Bilirubin

Bilirubin was assessed using the Diazo method (Wahlefeld et al., 1972). Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a diazonium ion in a strongly acidic medium (pH 1-2). The intensity of the colour of the asobilirubin produced is proportional to the total bilirubin concentration and is measured photometrically.

2.5.6 Kidney Measurements

Kidney function was assessed through measurements of creatinine in the blood as well as in the urine. Data sheets containing additional information on these analytes can be found as Appendix D. Both were measured using in vitro tests for their respective quantitative determinations in human serum, plasma, and urine on Roche/Hitachi cobas c systems, which automatically calculate the analyte concentration of each sample. Reference ranges and analyte testing methods were obtained via information sheets from cobas. These information sheets can be found as Appendix E. Serum and urine creatinine was determined through an enzymatic method based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde, and hydrogen peroxide. Catalyzed by peroxidase, the liberated hydrogen

peroxide reacts with the 4-aminophenazone and HTIB to form a quinone imine chromagen. The colour intensity of the quinone imine chromagen formed is directly proportional to the creatinine concentration in the reaction mixture.

2.5.7 Creatinine Clearance

Using both serum and urine creatinine measurements (from 24-hour urine collection) allows us to assess creatinine clearance (CrCl), which much more accurately reflects GFR and kidney function. CrCl is expressed as the amount of creatinine excreted per unit time (usually ml/min) and is determined using the following equation:

$$\text{Creatinine Clearance} = (\text{Urine Creatinine/Serum Creatinine}) \times (\text{Urine volume/time})$$

2.5.8 Liver and Kidney Adverse Events

The tests listed above were grouped for each organ (kidney or liver) and any individual test result that was outside the normal range was flagged as an adverse event. Participants who tested outside the reference range(s) for any measures at baseline were flagged and subsequent test results outside the reference range(s) were not counted unless they had returned to normal first. An adverse event for the same measure was also not counted twice if it occurred on subsequent tests. For example, if Participant #27 had an adverse event for AST at baseline and at 4 months, this would not be counted as an adverse event. If AST was elevated at baseline, returned to normal at 4 months, and was high again at 8 months this would be counted as an adverse event. If AST remained high at 12 months, it would still only be counted as one adverse event. Adverse events for different measures that occurred at the same time point e.g.) AST and ALT elevated at 4 months, were counted as separate adverse events. Lastly, values outside the normal range that

would not indicate a health problem, such as low liver enzyme levels, were not included as adverse events. Moderate elevations in blood and urine creatinine were also not included because these would be expected to increase during creatine supplementation due to the breakdown of creatine to creatinine. A kidney problem would be indicated by low creatinine clearance, where there is a buildup up of creatinine in the blood due to inadequate clearance by the kidneys, so blood creatinine is high relative to urine creatinine levels. Adverse event totals were compared between groups to give additional information on our collected data. Different conclusions would likely be drawn if many participants saw a moderate rise in observed measures as opposed to a few participants with extreme rises, even though this might have the same effect on the mean.

2.5.9 Other Adverse Events

Research assistants made inquiries about adverse events each time they had contact with participants. An adverse event form was used for causality assessment of the intervention (not related, unlikely, possibly, probably, definite), whether the adverse event was “serious” (i.e. resulted in death, life-threatening, required hospitalization, or resulted in persistent disability) or “non-serious”, and its intensity (mild, moderate, severe, life-threatening). The definitions for these are below, and follow ICH guidelines (ICH, 1994). Relationship to the intervention was determined by exploration of alternate causes and dechallenge/rechallenge of the supplement/exercise protocol used in previous research. AEs reported by participants initially recorded as “Not Related” or “Unlikely” depending on whether an alternative explanation could be established. If a temporal relationship but alternative explanation both existed, the AE was classified as “Possible” and tested using a dechallenge/rechallenge protocol. Dechallenge

involves removing the intervention (supplement/exercise) for a period of 1-2 weeks and monitoring the symptom(s). If the symptom(s) subside or disappear then dechallenge is confirmed and the relationship status is reclassified as “probable”. Rechallenge involves reintroducing the intervention once the symptom(s) have subsided. If they return upon reintroduction, then rechallenge is confirmed and the relationship status is reclassified as “definite”. Table 2-3 (below) provides a description of each relationship:

Table 2-3. Relationship of Adverse Events to Intervention

Relationship	Description/Determination
Not Related	AE is not related to intervention No temporal/suggestive relationship; alternative causal relationship established
Unlikely	AE is unlikely related to intervention Temporal relationship exists but alternative explanation more likely
Possible	AE may be related to intervention Temporal relationship suggestive; could not be confirmed by challenge/dechallenge
Probable	AE is likely related to the intervention Confirmed by dechallenge
Definite	AE is definitely related to the intervention/has no alternate reasonable explanation Confirmed by dechallenge and rechallenge

The participant using the following definitions rated intensity of adverse events:

Mild: Awareness of sign or symptom, but easily tolerated

Moderate: Discomfort enough to cause interference with normal daily activities

Severe: Inability to perform normal daily activities

Life Threatening: Immediate risk of death from the reaction as it occurred

Adverse events that have been associated with creatine supplementation in previous research (GI discomfort, cramping, etc.) were grouped together and tabulated over the course of the study as previous research had indicated a possible relationship between creatine supplementation and some of these side effects in older individuals (Chrusch et al., 2001). These adverse events were compared across groups once the study was complete to assess a possible relationship.

2.6 Statistical Analysis

A group x time (2x4) mixed ANOVA was used to determine differences both between and within groups for all liver and kidney measurements over the course of the study. Significant main effects were tested post hoc using the Pairwise method and Bonferroni adjustment to determine which means were significantly different. Where sphericity violations occurred, Greenhouse-Geisser correction was used. Missing data points were replaced using the last observation carried forward (LOCF) to retain participant numbers vs. listwise deletion, which is the default for SPSS. This method is commonly used in pharmaceutical and longitudinal research. Group mean was substituted in instances where last observation was not available. All ANOVAs and post hoc tests were performed using SPSS 20.

Chi-square analysis was performed to determine any significant difference between the number of adverse events for liver measurements, kidney measurements, and GI/cramping related adverse events between groups over the course of the study. Chi-square analysis performed using

online tool via quantpsy.org (Preacher, 2001). Statistical significance was set at $\alpha=0.05$. Data are expressed as mean (SD) unless otherwise indicated.

3. RESULTS

3.1 Liver Measures

3.1.1 Aspartate Aminotransferase (AST)

Mauchly's Test of Sphericity was significant $W(5) = 0.620$, $p < .05$, therefore sphericity cannot be assumed. Analysis of variance revealed a significant main effect of time on AST across group.

$F(1,914,130.141) = 9.550$, $p < 0.001$, $\eta_p^2 = 0.123$ but no significant main effect of group on AST across time. $F(1,68) = 2.410$, $p = 0.125$, $\eta_p^2 = 0.034$ and no significant interaction of time and group. $F(1,68) = 0.448$, $p = 0.505$, $\eta_p^2 = 0.007$.

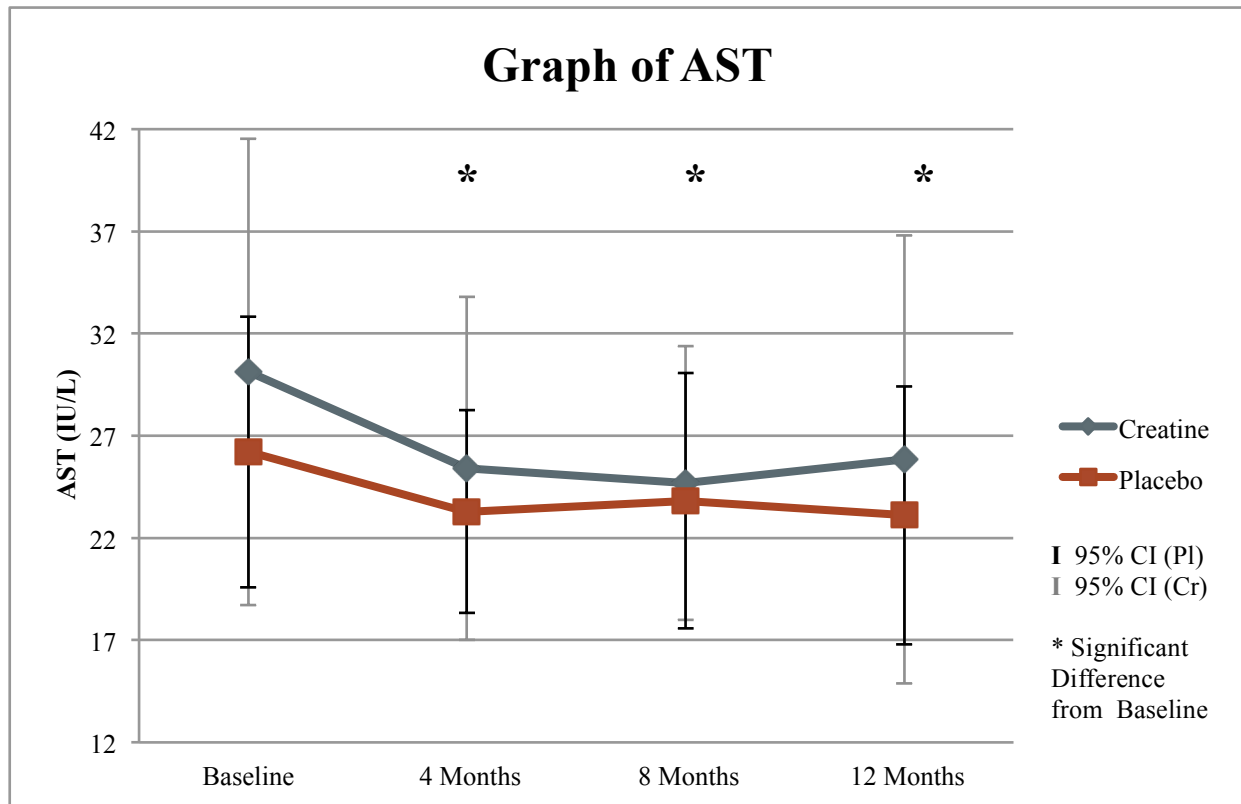


Figure 3-1. Graph of AST

AST levels in both groups decreased significantly over the course of the study. Post hoc revealed that AST levels decreased significantly between baseline and 4 months ($p=0.003$), remained significantly lower than baseline at 8 months ($p=0.001$) and 12 months ($p=0.016$).

The effect size ($\eta_p^2 = 0.034$) was quite small between the two groups and there was no significant interaction. Mean AST for both groups also remained within the normal range (10-34IU/L) for the duration of the study.

3.1.2 Alanine Aminotransferase (ALT)

Mauchly's Test of Sphericity was significant $W(5) = 0.556$, $p < .05$, therefore sphericity cannot be assumed. There was a significant main effect of time on ALT. $F(2.222, 151.065) = 8.506$, $p < 0.001$, $\eta_p^2 = 0.111$ across group but no main effect of group on ALT pooled across time $F(1, 68) = 2.645$, $p = 0.109$, $\eta_p^2 = 0.037$. There was also no significant interaction of time and group, $F(1, 68) = 2.643$, $p = 0.109$, $\eta_p^2 = 0.037$.

ALT values for both groups decreased from baseline to 4 months. While this difference only approached significance $p = 0.052$, it became significant at 8 months $p = 0.003$ and remained significantly lower at 12 months $p = 0.002$. The effect size was small $\eta_p^2 = 0.183$, there was no significant interaction, and both were still within the normal range (10-40IU/L).

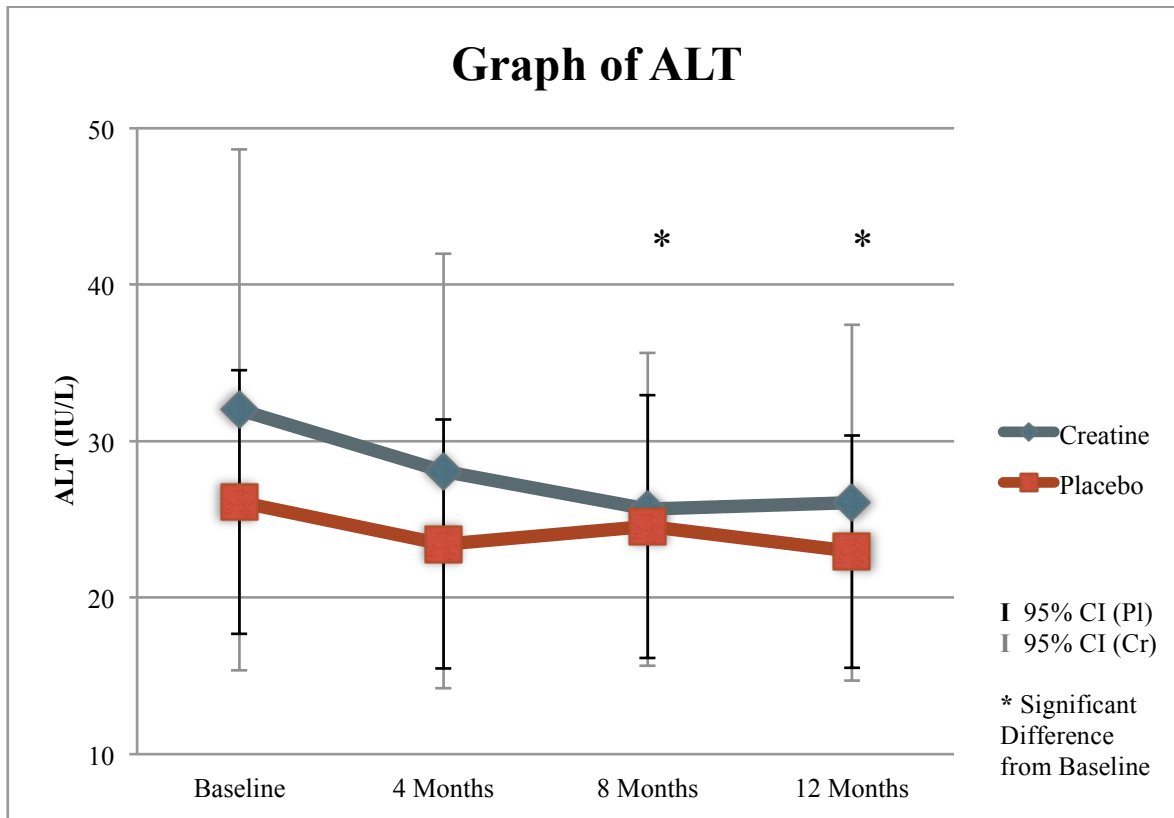


Figure 3-2. Graph of ALT

3.1.3 Alkaline Phosphatase (ALP)

Mauchly's Test of Sphericity was significant $W(5) = 0.496$, $p < .05$, therefore sphericity cannot be assumed. There was a significant main effect of time $F(2.164, 147.153) = 10.017$, $p < 0.001$, $\eta_p^2 = 0.128$ on ALP, but no main effect of group $F(1, 68) = 0.059$, $p = 0.809$, $\eta_p^2 = 0.001$ and no significant interaction of time and group $F(2.164, 147.153) = 1.652$, $p = 0.179$, $\eta_p^2 = 0.024$.

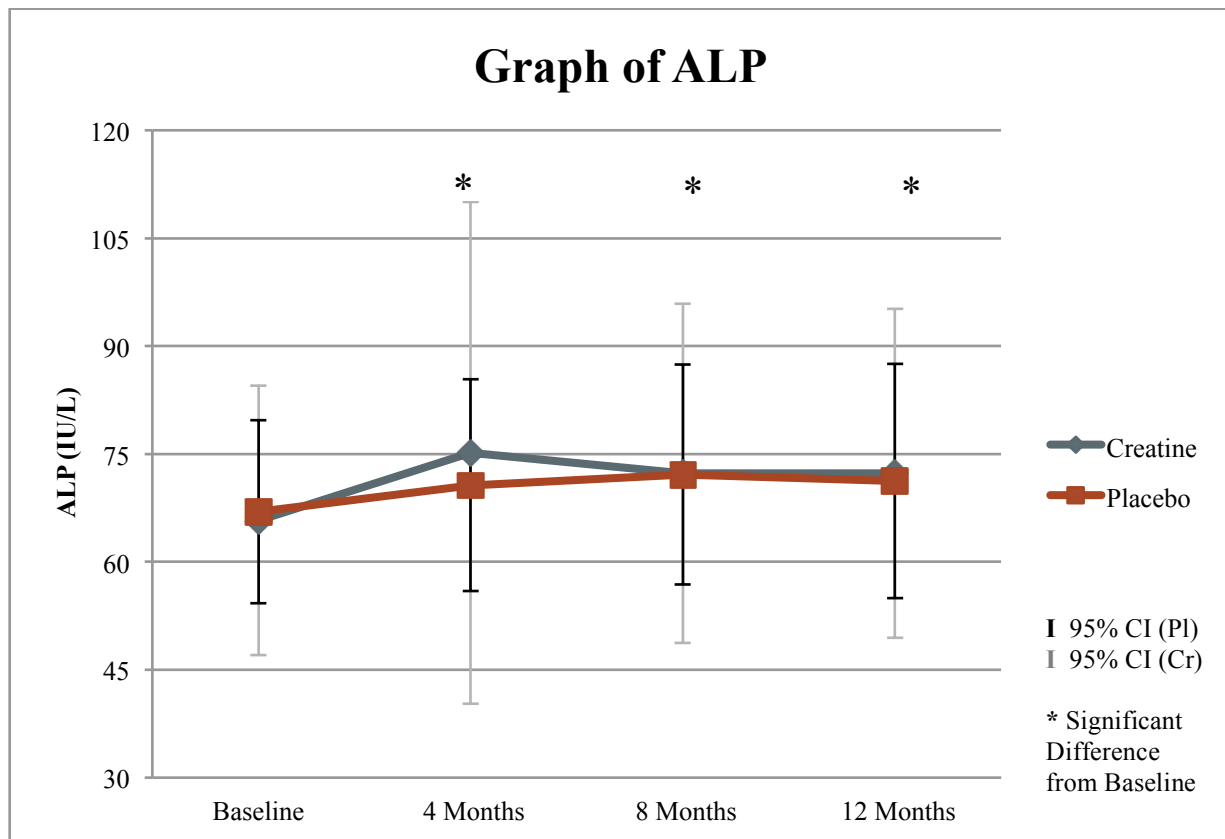


Figure 3-3. Graph of ALP

ALP increased significantly in both groups over the course of the study. Post hoc revealed that ALP increased significantly between baseline and 4 months $p = 0.002$ and remained significantly higher than baseline at 8 and 12 months $p < 0.001$. The effect size for this difference was relatively small, $\eta_p^2 = 0.128$, and both groups' ALP levels also remained well within the normal range (35-130IU/L) at all time points.

3.1.4 Bilirubin

Mauchly's Test of Sphericity was significant $W(5) = 0.803, p < .05$, therefore sphericity cannot be assumed. There was a significant main effect of time on Bilirubin across groups.

$F(2.635, 179.187) = 27.097, p < 0.001, \eta_p^2 = 0.285$. There was not a significant main effect of group on Bilirubin pooled across time. $F(1, 68) = 0.000, p = 0.983, \eta_p^2 = 0.000$ or a significant interaction of time and group. $F(2.635, 179.187) = 1.016, p = 0.380, \eta_p^2 = 0.015$.

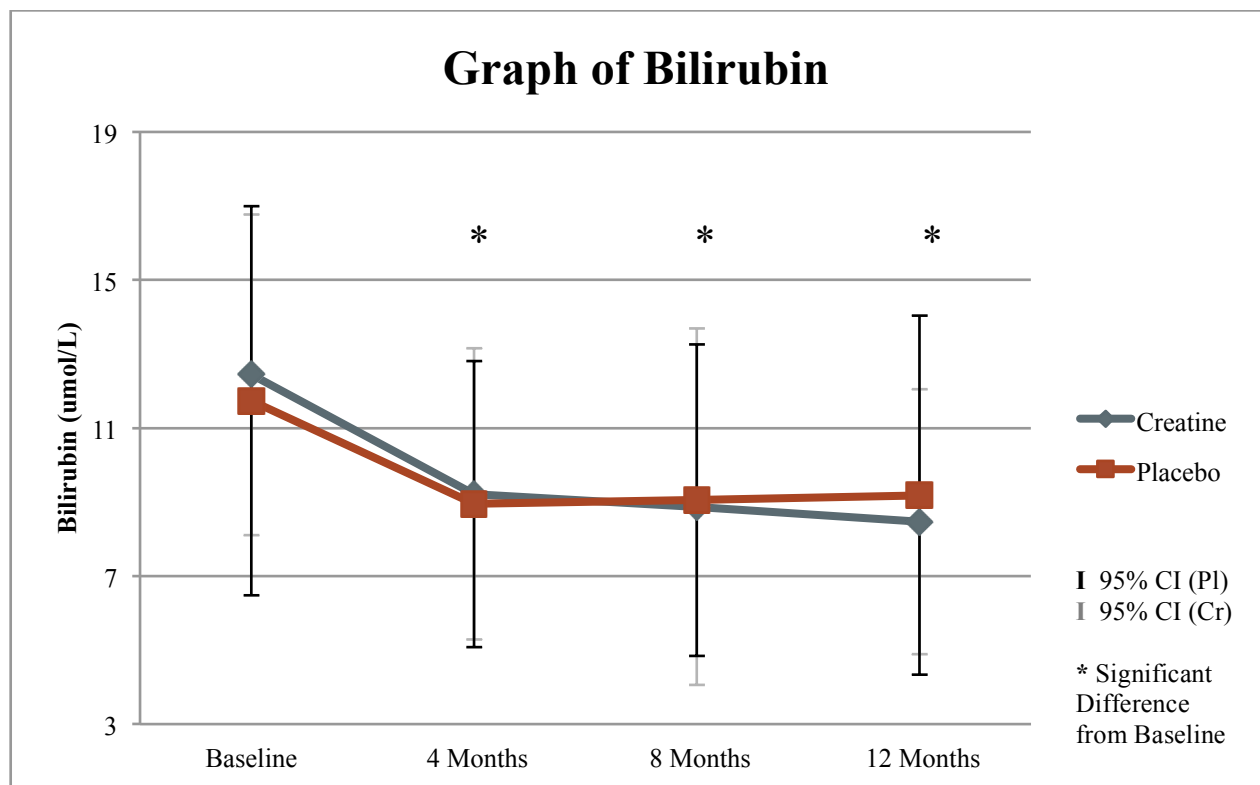


Figure 3-4. Graph of Bilirubin

Bilirubin levels significantly decreased over the course of the study for both groups. Post hoc revealed that mean bilirubin significantly decreased from baseline to 4 months ($p < 0.001$), and remained significantly lower than baseline at 8 and 12 months ($p < 0.001$). The effect size was the

largest of all tested variables, but was still relatively small $\eta_p^2 = 0.285$, and bilirubin levels in both groups at all timepoints remained within the normal range (up to 21 $\mu\text{mol/L}$).

3.1.5 Liver Adverse Events - Chi-Square Analysis

Chi-square analysis of liver adverse events revealed no significant difference in adverse events reported by group $\chi^2(1, N=11) = 0.091, p=0.763$ and the difference remained statistically not significant when Yates' corrected $\chi^2(1, N=11) = 0.000, p=1.000$.

Table 3-1. Liver Adverse Events

Group	Total AEs	Timepoint (# AEs)	Type (#)
Creatine	5	4 months (2)	High AST, High ALT
		8 months (1)	High Bilirubin
		12 months (2)	High AST, High ALT
Placebo	6	4 months (1)	High Bilirubin
		8 months (3)	High AST, High ALT, High ALP
		12 months (2)	High AST, High Bilirubin

3.2 Kidney Measures

3.2.1 Serum Creatinine

Mauchly's Test of Sphericity was significant $W(5) = 0.777, p<.05$, therefore sphericity cannot be assumed. There was a significant main effect of time $F(2.588, 175.955) = 2.887, p = 0.045, \eta_p^2 = 0.041$ across group, but there was no significant effect of group across time $F(1, 68) = 1.378, p =$

0.245, $\eta_p^2 = 0.020$ on serum creatinine. There was also no significant interaction of time and group $F(2.588, 175.955) = 2.267$, $p = 0.092$, $\eta_p^2 = 0.032$.

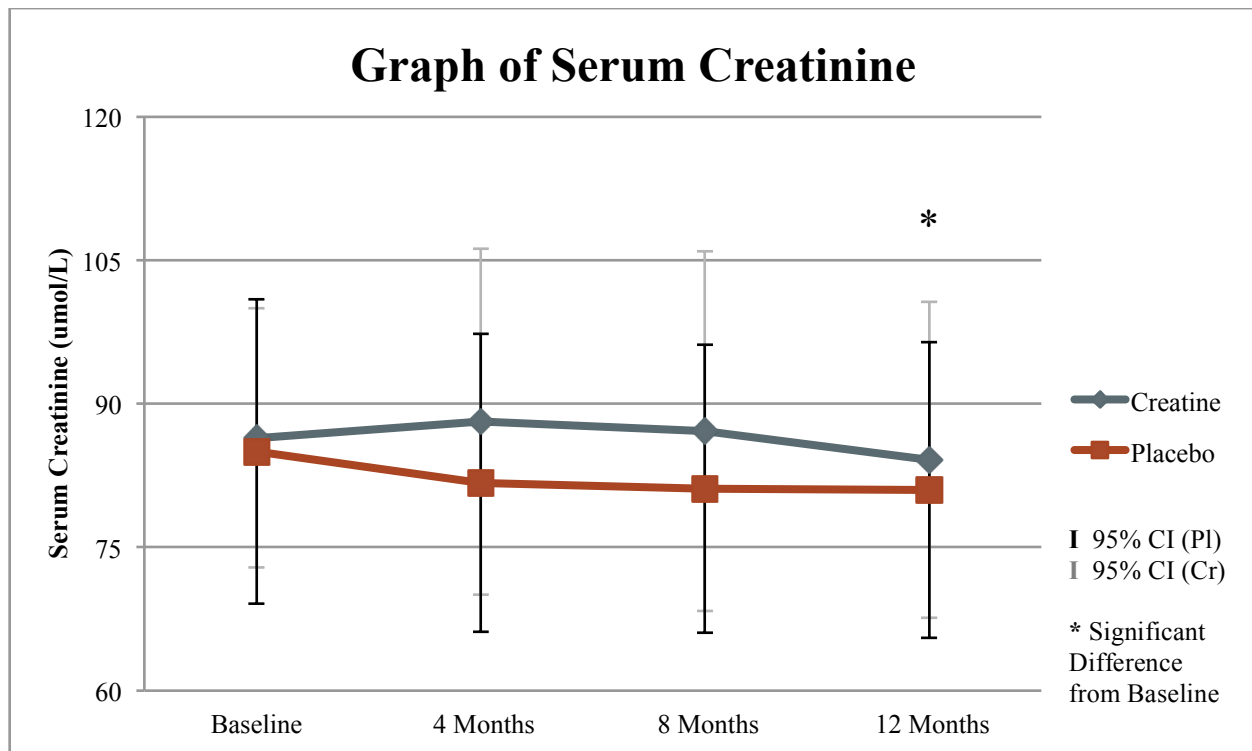


Figure 3-5. Graph of Serum Creatinine

Both groups saw a significant decrease in serum creatinine over the course of the study. Post hoc revealed this difference was only significant between baseline and 12 months $p = 0.045$ and the effect size was quite small $\eta_p^2 = 0.041$.

3.2.2 Urine Creatinine

There was no significant main effect of time $F(3,204) = 2.093$, $p = 0.102$, $\eta_p^2 = 0.030$ or group $F(1,68) = 1.782$, $p = 0.186$, $\eta_p^2 = 0.026$ on urine creatinine. There was also no significant interaction of time and group. $F(3,204) = 1.976$, $p = 0.119$, $\eta_p^2 = 0.028$.

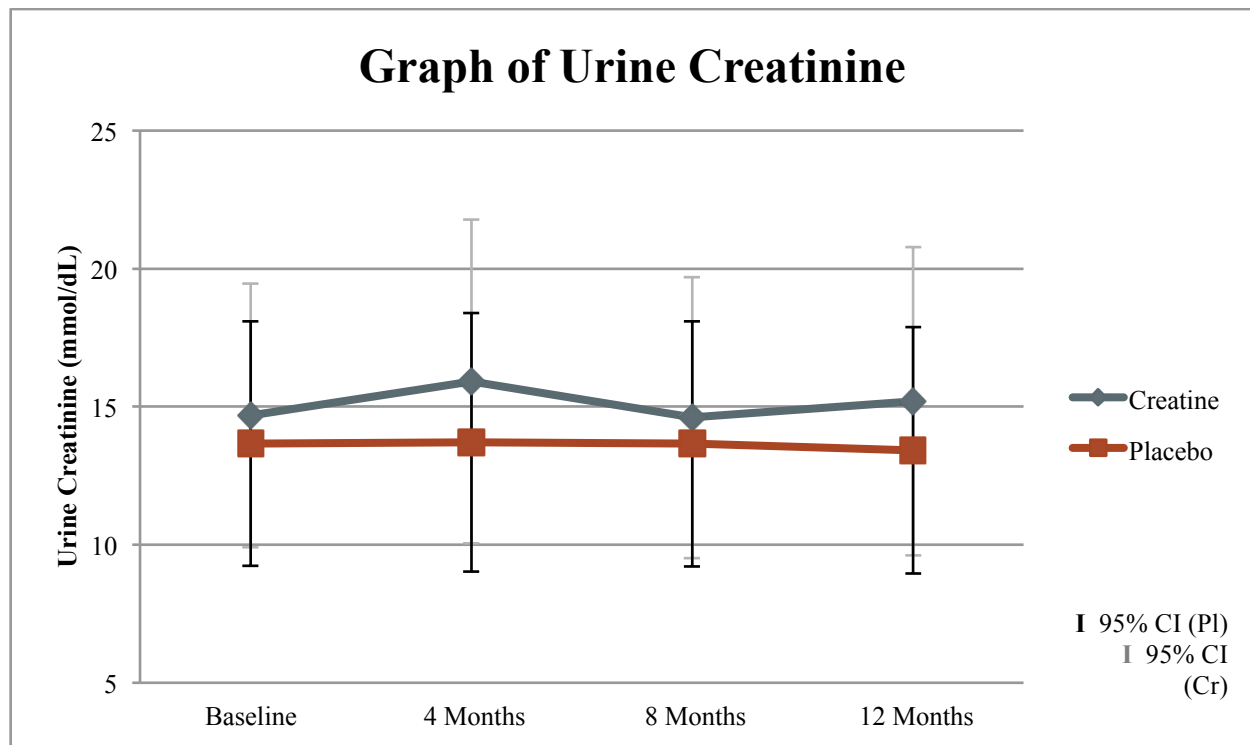


Figure 3-6. Graph of Urine Creatinine

Urine creatinine, while not significantly different between groups, did appear to indicate a trend towards being different, with p levels being between 0.1 and 0.2 for both main effects and the interaction. Using a more liberal α , a significant difference could have been seen, even though the effect sizes were very small, and values for both groups stayed within the normal range (7.5-16mmol/L) throughout the study.

3.2.3 Creatinine Clearance

Mauchly's Test of Sphericity was significant $W(5) = 0.802, p < .05$, therefore sphericity cannot be assumed. There was a significant main effect of time $F(2.597, 176.601) = 4.221, p = 0.009, \eta_p^2 = 0.058$, but no main effect of group $F(1, 68) = 0.588, p = 0.446, \eta_p^2 = 0.009$ on creatinine clearance. There was also no significant interaction of time and group. $F(2.597, 176.601) = 1.120, p = 0.338, \eta_p^2 = 0.016$.

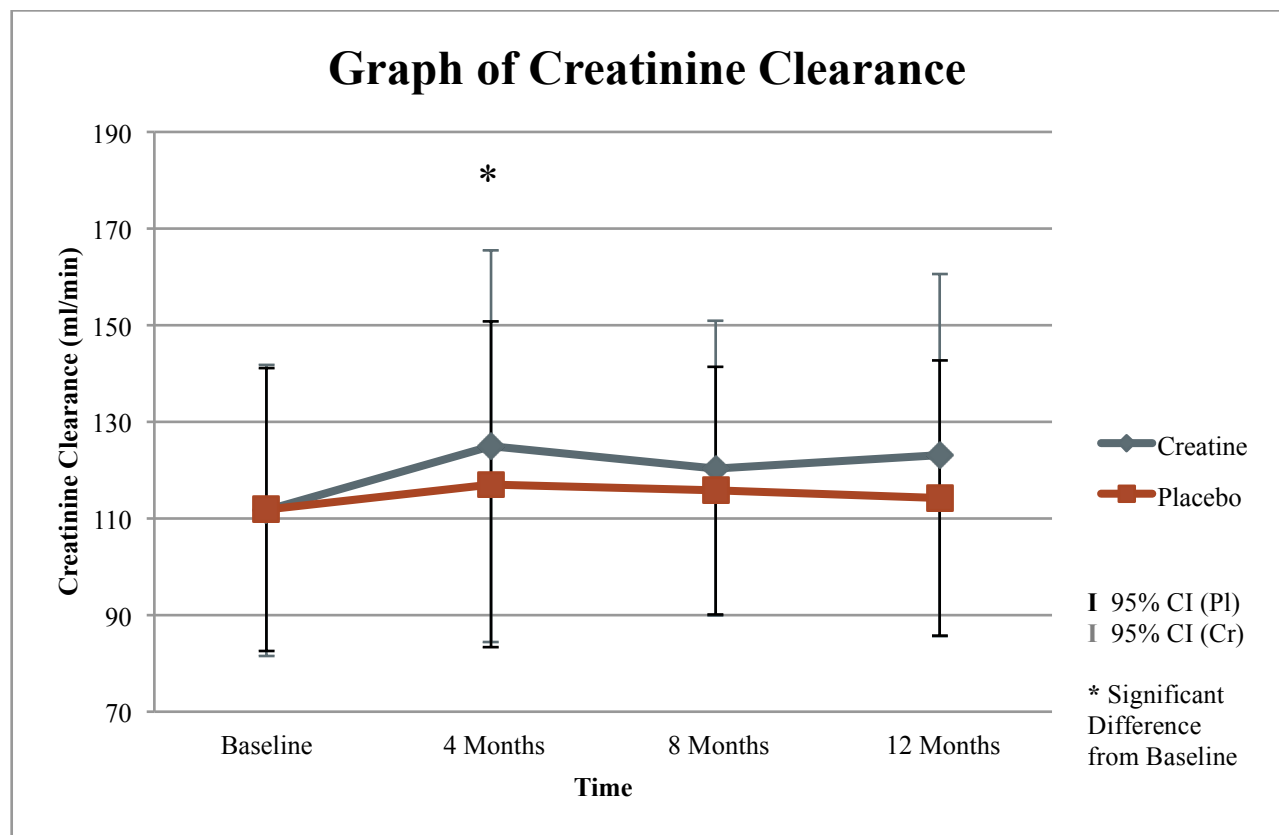


Figure 3 – 7. Graph of Creatinine Clearance

There was a significant increase in the creatinine clearance of both groups during the study. Post hoc revealed a significant increase in creatinine clearance from baseline to 4 months $p = 0.003$,

but this difference became not significant at 8 months $p = 0.166$ and remained not significant at 12 months $p = 0.091$. The effect size was very small $\eta_p^2 = 0.058$, and the means for both groups at all time points remained within the normal range for healthy adults (93-133ml/min).

3.2.4 Kidney Adverse Events - Chi Square Analysis

There was only 1 adverse event related to kidney function found in either group: creatine = 0, placebo = 1 (low creatinine clearance). Chi-square analysis was not performed due to the extremely low number of adverse events (one total) over the course of the study.

3.3 Other Adverse Events

3.3.1 Chi-Square Analysis

Chi-square analysis of reported adverse events that have been thought to be linked to creatine supplementation (GI distress, cramping, etc.) revealed a significant difference in adverse events reported by group $\chi^2(1, N=22) = 4.55, p=0.033$ although the difference was not significant when Yates corrected: $\chi^2(1, N=22) = 3.68, p=0.055$. This suggests that there could be a link between creatine supplementation and these types of side effects that have occurred in other studies previously. The frequency and type of each adverse event is listed in Table 3-2 on the next page:

3.3.2 Summary/Conclusions

There was a significant difference between the total number of other AEs in the creatine vs. placebo groups, which indicates creatine supplementation may contribute to side effects such as diarrhea and muscle cramps.

Table 3-2. Other Adverse Events

Group	Total AEs	Type (#)	Intensity (#)	Related (#)
Creatine	16	Indigestion (1), Heartburn (1) Nausea (1) Stomach Pain (1), Cramps (1) Constipation (1) Diarrhea (5) Muscle Cramping (5)	Mild (2) Mild Mild (2) Mild Mild (5) Mild (4), Moderate (1)	Possible (2) Possible Possible, Probable Possible Probable (1), Possible (4) Possible
Placebo	6	Indigestion Nausea (2) Bloating Muscle Cramping (2)	Mild Mild (2) Mild Mild (2)	Possible Unlikely (1), Definite (1) Possible Possible (2)

4. DISCUSSION

4.1 Summary of Important Results

Results of the present study showed significant changes in all measures over time except for urine creatinine, although none of these showed significant differences between groups nor did any show an interaction between time and group. AST and Bilirubin significantly decreased from baseline to 4 months and remained significantly lower than baseline at 8 and 12 months. ALT decreased between baseline and 4 months, but this difference was not significant until 8 and 12 months, while SCr was not significantly lower than baseline until 12 months. ALP significantly increased from baseline to 4 months and remained significantly higher than baseline at 8 and 12 months while CrCl significantly increased from baseline to 4 months but the difference was not significant at 8 and 12 months.. AST, ALT, and UCr all showed trends towards a main effect of group ($p = 0.125, 0.109, \text{ and } 0.186$) but these were not significant.. The differences found between the frequency counts of Adverse Events (AEs) related to liver function and kidney function between groups were also not significant. Importantly, we did discover a significant difference in the frequency counts for GI and muscular cramping related AEs between groups (creatine: 16 events; placebo: 6 events; $p < 0.05$). The creatine group reported indigestion (n=1), heartburn (n=1), nausea (n=1), stomach pain/cramps (n=2), constipation (n=1), diarrhea (n=5), and muscle cramping (n=5); whereas the placebo group reported indigestion (n=1), bloating (n=1), nausea (n=2), and muscle cramping (n=2). Most of these AEs were rated as “mild” in intensity; one muscle cramping AE in the placebo group was rated as “moderate”.

An unexpected finding was that SCr and three liver measures (AST, ALT, and Bilirubin) dropped in both groups over the course of the study. This was unexpected, as previous literature

has shown acute increases in liver enzyme levels with resistance exercise (Nathwani et al., 2005; Tarnopolsky, 2010). The fact that some of these decreases were statistically significant, but did not have a group x time interaction, could indicate possible positive effects of resistance exercise on liver health in the long-term. This theory is supported by results from Souza et al. (2005), who found increased liver enzymes (AST and ALT) in sedentary rats but not exercised rats supplementing with creatine. Excitement may be premature though, as all levels still remained within normal ranges, and drastic changes in liver enzyme levels are usually required to indicate decline in liver health, not to mention moderate variations in liver enzymes are not often accurate indicators of liver function.

These results indicate that creatine supplementation of 0.1g/kg/day in older adults over 12 months may not adversely affect liver or kidney health, although strong conclusion statements are difficult to draw due to the study's lack of power. The results also indicate creatine supplementation of this duration and dosage can significantly increase mild to moderate side effects related to GI symptoms and muscle cramping.

4.2 Results versus Hypotheses

The results of this study support the hypothesis that creatine supplementation of 0.1g/kg/day dosage in an older population over a twelve month period will not significantly increase markers of liver or kidney dysfunction (Figures 3-1 to 3-7, Table 3-1), although the protocol used can significantly increase ALP and CrCl. The hypothesis that the frequency of adverse events related to liver and kidney function reported would not be significantly different was also supported by our results. These findings add credibility to the claim that creatine supplementation may be safe

within this population over the long term, but additional, more powerful studies are needed to strengthen this assessment.

Our results did find a significant difference in the frequency of adverse events related to GI symptoms and muscle cramping reported (Table 3-2), thus forcing us to conclude that creatine supplementation at this dose and in this population can contribute to an increased incidence of these side effects.

4.3 Study Strengths

This is the only study to measure the effects of creatine supplementation and resistance exercise on kidney and liver function in an older population over a twelve-month period. There have been studies done on older adults (Neves et al., 2011; Candow et al., 2008; Chrusch et al., 2001) and in the long-term (Poortmans et al., 1999; Schroder et al., 2005), but very few that have studied older adults over the long term (Groeneveld et al., 2005; Bender et al., 2008). Both of these studies observed specific older populations (adults with ALS and Parkinson's Disease respectively) and did not incorporate resistance training, which has been shown to influence some of the hepatic and renal outcome measures associated with creatine supplementation in rats (Souza et al., 2009). Many researchers (Kim et al., 2011; Juhn & Tarnopolsky, 1998) have observed the need for long term, randomized, placebo-controlled studies in the literature to more accurately assess the effects of creatine supplementation in this population and time period. The current study adds to this lacking, but slowly growing research area.

4.3.1 Creatinine Clearance

Measurements of serum creatinine can be used in predictive equations to determine GFR and thereby ascertain kidney function. This method is very accurate, but contraindicated in

individuals supplementing with creatine due to the nature of creatine metabolism, which is spontaneously converted to creatinine following ingestion (National Kidney Foundation), thus inflating this measure in the absence of renal dysfunction. Creatinine clearance (CrCl), determined by 24-hour urine collection and serum levels of creatinine, is a valuable alternative, and may provide better estimates of GFR than predictive equations and serum creatinine in individuals supplementing with creatine (National Kidney Foundation). Other researchers (Gualano et al., 2008) have noted difficulty in using CrCl as 24 hour urine collection is required and participant compliance for this measure can be unreliable. The length of the current study made compliance less onerous as participants were only required to perform this measure four times, with each collection spread over several months.

Even though the differences were not statistically significant, the creatine group's urine creatinine and creatinine clearance levels increased by 3.4% and 7.6% over the course of the study (vs. -1.5% and 2.2% respectively in the placebo group). This seems to indicate that the supplement was being metabolized, perhaps just not in large enough quantities to have a significant effect on these measures. It is suggested that creatinine excretion is proportional to muscle mass (Tarnopolsky, 2010) and thus decreases with age. This indicates urine creatinine levels, and therefore creatinine clearance, would likely be lower in an older population and may not have been affected by creatine supplementation in this study as much as those observed in studies involving younger populations. Some other researchers, however, found no difference in urine creatinine levels between old and young participants supplementing with creatine (Rawson et al., 2002), although the duration of that study was only one week.

4.3.2 Liver Measures

The number of measures taken indicative of liver health is also a strength of the current study. Previous research has indicated that ALT and AST levels may not be accurate representations of hepatic function in an environment with elevated creatine kinase activity because both of these enzymes exist in high levels within skeletal muscle (Tarnopolsky et al., 2010). The present study also measured serum Bilirubin and ALP levels in an effort to strengthen any changes that might be seen in liver enzyme activity as a result of long-term creatine supplementation. Procedures for procuring and testing samples were also well established and controlled by medically trained personnel, because these serum measurements are also part of a standard medical blood panel.

4.4 Study Limitations

The number of participants in the current study may have limited the analysis. Urine creatinine and creatinine clearance both increased to a greater extent in the creatine group vs. the placebo group but these increases were not significant. Sample size calculations showed a much larger participant pool would have been needed for this difference to be significant (161 and 229 per group respectively). Calculations to determine main effect differences for group showed that 300-400 participants would have been required to see significant differences between groups for AST, ALT, SCr, UCr, and CrCl. Related to participant number is retention of participants. 210 individuals were initially recruited for the study and only 70 participants met inclusion criteria and were randomized. Over the course of the study several dropped out, and 20 missed at least one measurement of the 4 that were taken. The LOCF method to substitute missing data points was implemented, so that pairwise deletion was avoided and sample size could be preserved. This method is commonly used in pharmaceutical and longitudinal research but obviously it

would have been better to have the actual measurements from participants. Another limitation related to statistical analysis was the intent to treat analysis method. A stricter per protocol analysis would have been more sensitive to change in participant groups and may have allowed significant differences to be seen.

It has also been shown that muscle PCr concentrations are influenced to a greater degree by creatine supplementation in younger populations, versus older, (Rawson, et al., 2002). While this is mostly attributed to differences in muscle mass, it's possible that an absorption/metabolism issue could also be at work. Both these factors could contributed to the effects of creatine supplementation in the present study being attenuated by the age of the participants. While this was unavoidable given that we were investigating this population specifically, it should be noted due to its likely effects on the outcome measures.

4.5 Previous Literature

Direct comparisons are difficult given that this was one of the only studies to use creatinine clearance as a measure of kidney function; most other studies used serum creatinine, sometimes combined with predictive equations to estimate GFR. Our results were consistent with research showing no adverse effects of creatine supplementation on renal function (Gualano et al., 2008; Groeneveld et al., 2005; Poortmans et al., 1999) and liver enzyme activity (Tarnopolsky et al., 2008; Kreider et al., 2003) and was in contradiction of literature which showed adverse effects of creatine on these systems (Keys et al., 2001; Duarte et al., 1999; Yu&Deng 2000; Pritchard & Kalra 1998).

4.5.1 Creatine and Liver Function

The effects of creatine supplementation on liver function have been fairly consistent. Aside from one study which showed elevated liver transaminases in football players supplementing with creatine (Kreider et al., 1998) and another which showed liver inflammation in mice given creatine monohydrate (Tarnopolsky, 2003), evidence supporting the claim that creatine supplementation may damage the liver is extremely sparse. The reason postulated that some research has found evidence that creatine supplementation has adverse effects on liver function is a reliance on measures of AST and ALT, which have been shown in the literature to become elevated under conditions of skeletal muscle damage consistent with resistance training (Saengsirisuwan et al., 1998; De Paz et al., 1995; and Fallon et al., 1999). These results are supported by another study that showed elevated AST and ALT levels in patients with muscular necrosis (Nathwani et al., 2005). It has been suggested that serum AST and ALT levels may not be reliable indicators of liver function in situations where creatine kinase levels are elevated, for example during resistance exercise, caffeine supplementation, etc (Tarnopolsky, 2010). Studies supporting the theory that creatine supplementation has no adverse effects on liver function, used γ -glutamyltransferase and bilirubin to measure liver stress and found no significant increases in groups supplementing with creatine (Tarnopolsky et al., 2008, Kreider et al., 2003). Conclusions drawn from these studies are extremely valuable when reviewing our data, because the majority of liver related AEs in both groups were AST and ALT (4/5 in the creatine group and 3/6 in the placebo group). The conclusion that liver function is not affected by creatine supplementation is strengthened by the ANOVA data, which showed three of the four liver measures (AST, ALT, and Bilirubin) significantly decreased during the study. Increased levels of AST are associated

with liver damage/disease. Increased Bilirubin levels are also associated with liver stress, so while a decrease during the study cannot be definitively labelled a health benefit, it would seem to indicate no negative effects, especially considering the levels of both groups still remained within the normal range for healthy adults (up to 21 $\mu\text{mol/L}$).

It is possible the exercise intervention was responsible for this decrease in the form of a training adaptation, as neither supplement is known to cause decreased AST, ALT, or Bilirubin on its own, but some research has shown increased ALT and AST in sedentary rats vs. exercised rats both supplementing with creatine (Souza et al., 2009). The increase in ALP seen is also likely due to bone activity rather than an indication of liver stress because of what was seen in the other liver measures, but this should be explored in future research. ALP levels in both groups and at all time points were well within the normal range of 35-130 IU/L. The ANOVA data also showed no group x time interactions, indicating that even the significant changes in some variables occurred in the same way for both groups so it's more likely they occurred due to the exercise intervention, which both groups performed, as opposed to the different supplement taken. The lack of evidence in the literature, combined with the present research, makes it seem likely that creatine supplementation does not adversely affect liver function, but because of the liver's role in creatine metabolism it is prudent to observe liver function in individuals supplementing with creatine, especially those in populations at risk of liver complications such as older adults.

4.5.2 Creatine and Kidney Function

The kidneys' role in creatine metabolism is well known, and the possible adverse effects of creatine supplementation on kidney function gained mainstream attention following the publication of two studies which showed improvement of kidney function in two individuals with kidney disease following the termination of creatine supplementation (Pritchard & Kalra, 1998; Koshy et al., 1999). Research has also shown increased cyst growth and a decline in renal function of rats supplementing with creatine (Edmunds et al., 2001). Since then, many studies have been done which indicate no harmful renal effects of creatine supplementation in humans (Poortmans & Francaux, 1999; Gualano et al., 2008; Tarnopolsky, 2010). So while much of the more current research finds no adverse effects of creatine supplementation on renal function, the total body of research on the subject remains mixed.

The current study measured serum creatinine (SCr), urine creatinine (UCr), and creatinine clearance (CrCl) to thoroughly assess kidney function over the course of twelve months. Both groups saw a significant decrease in SCr between baseline and 12 months, which would seem to indicate that they were not able to maintain their creatine stores over time. This is surprising, because creatinine is a byproduct of creatine metabolism, and so a group supplementing with creatine would be expected to have a higher level of serum creatinine than a placebo group, not to mention we would have thought the creatine group would maintain their creatine levels due to the supplement protocol. While the decrease was significant, the means for both groups remained within the normal range for healthy adults (45-110 $\mu\text{mol/L}$) over the course of the study.

Levels of urine creatinine behaved slightly differently between groups, with the creatine group's level slightly increasing and the placebo group's level slightly decreasing over the course of the

study. This difference was not significant, likely due to the high amount of variance seen in the measurement data. A difference would make sense between groups because excess creatinine is excreted in the urine, thus creatine supplementation would likely cause an increase in urine creatinine levels. Because none of the differences were significant and the means for both groups remained within the normal range for healthy adults of 7.5 – 16 mmol/L (Junge et al., 2004) at nearly all time points, the evidence suggests that creatine supplementation does not have a significant effect on urine creatinine levels at the tested dosage. It is possible that a significant effect could be seen at higher dosages or with greater participant numbers though, as there appeared to be a trend toward a difference ($p=0.081$). A significant difference in this measure would likely not have affected our conclusions though, as an increase in urine (excreted) creatinine would be expected in a group supplementing with creatine.

Both groups saw a significant increase in CrCl between baseline and 4 months, but this difference was not statistically significant at 8 and 12 months. The increase appeared greater in the creatine group, which suggests that at higher dosages this effect could become significant. This would be expected, as the increased creatine ingestion would cause an increase in creatinine excretion, assuming cellular saturation of creatine. It is also for this reason that increased creatinine clearance is not a health concern, as it points to increased excretion. In instances of chronic and acute kidney disease, creatinine clearance is reduced, and neither experimental group experienced this. Further research is required in this area to ascertain the effect(s) creatine supplementation may have on creatinine clearance.

Overall, there is little evidence to suggest creatine supplementation of this amount and time, within this population has any adverse effects on kidney health. Serum creatinine significantly decreased, urine creatinine was not statistically different, and creatinine clearance increased only significantly between baseline and 4 months before returning to normal. Even if all these measures did significantly increase, this would not be unexpected in a group supplementing with creatine, as its metabolism would cause an increase in the byproducts (creatinine) to be present. The decrease in serum creatinine suggest that neither group was able to maintain their creatinine levels and thus future research should likely experiment with higher creatine dosing protocols to ensure tissue saturation, although caution should be exercised when supplementing with creatine, especially in populations with potential renal complications or at higher dosages. Future research should explore these avenues to more accurately define safe and potentially unsafe supplementation dosages on renal function.

Even though adverse effects of creatine supplementation on renal and liver function remain to be proven, creatinine (the byproduct of creatine) is primarily filtered and excreted by the kidneys. It will continue to be necessary to test and observe the effects creatine supplementation has on the renal system in the event that evidence of adverse reactions simply hasn't been discovered yet.

4.5.3 Creatine and Cramping

Previous studies observing effects of creatine supplementation on muscle cramping have been inconsistent. A recent review (Kim et al., 2011) concluded there was insufficient evidence to support the theory that creatine supplementation causes an electrolyte imbalance and leads to muscular cramping. It has also been theorized that there is an increased hydration demand while supplementing with creatine, because creatine supplementation increases intracellular water, and

that this could contribute to muscular cramping, although there is no evidence to support this claim in the literature (Lopez et al., 2009). A review done in 2008 (Dalbo et al.) concluded that not only was there no reason to believe that creatine supplementation would increase the risk of dehydration or muscle cramps, but that creatine can increase total body water, lower core body temperature, and reduce exercise heart and sweat rate, thereby decreasing the risk of dehydration when exercising. Our results showed a 150% increased incidence of muscle cramping in those supplementing with creatine, so perhaps in certain populations (such as older adults) the hydration demand of creatine is greater, or perhaps the increased workload the participants supplementing with creatine were able to undertake contributed to cramping. Another study examining the effects of creatine supplementation and resistance training in older adults (Chrusch et al., 2001) found a significant increase in muscle cramping in participants supplementing with creatine. This result is in contradiction to other studies, which have found no significant incidence of muscle cramping with creatine supplementation (Dalbo et al., 2008, Greenwood et al., 2003, Vandenburg et al., 1997).

Other studies have shown significant increases in training volume/intensity with creatine supplementation (Candow et al., 2008) vs. placebo, indicating that creatine may allow participants to push harder in training, thus increasing their risk of cramps, pulls, and strains. Due to the mixed results and inconsistent reporting of these types of AEs, continued attention in future research will be necessary to determine the nature of the relationship between them and creatine supplementation.

4.5.4 Creatine and Gastrointestinal (GI) Distress

In a similar fashion to muscle cramping incidents, reports of GI distress while supplementing with creatine are inconsistent. Some studies show an incidence rate for GI related issues in over 30% of participants (Juhn et al., 1999; Vandenburg et al., 1997), while others report no GI related disturbances at all (Kreider et al., 1998; Greenhaff et al., 1998). Several sources have postulated that GI distress observed in those supplementing with creatine is a result of the supplement not having completely dissolved before ingestion and that this problem could be avoided by either mixing the supplement in a more viscous medium (yogurt) and/or taking it in conjunction with a meal (Kim et al., 2011; Tarnopolsky, 2010). It should be noted that we heeded advice of the previous literature and instructed our participants to ingest their respective supplement with a meal on non-exercise days in an effort to minimize GI distress. The Tarnopolsky (2010) study cited a 5% incidence rate for GI disturbances while ours were 29% (GI) and 14% (cramping) in the creatine group vs. 11% (GI) and 6% (cramping) in the placebo group. These findings lend credibility to previously anecdotal claims of GI related disturbances occurring with creatine supplementation (Juhn et al., 1999; Lopez et al., 2009) and refute other research, which found no difference in GI symptom incidence between creatine and placebo groups (Groeneveld et al., 2005; Juhn et al., 1999; Vandenburg et al., 1997). Interestingly, the Groeneveld study showed an incidence of GI related symptoms as high as 35% (11% higher than the placebo group) but this difference was not found to be significant. Additional research must be performed to ascertain the nature of the relationship between creatine supplementation and these side effects, but in the meantime anyone planning to supplement with creatine should be

made aware of these possible side effects.

4.6 Future Directions

The present research indicates no serious risk to liver health at the tested dosage in the observed population. While it is also unlikely that creatine supplementation adversely affects kidney function, all our measurements of creatinine showed higher levels in the creatine vs. placebo groups, so renal involvement cannot be ignored and should continue to be assessed in future research, especially in populations that may be at increased risk for kidney disease. Future studies should experiment with higher dosages to ensure optimal creatine loading has occurred, as this was not evident in the current study. For example, serum creatinine was not maintained in either group over the course of the study. While this decrease was not significant in either group, it would be expected that optimal creatine loading would maintain or increase creatinine levels in the body. If this was not accomplished in the creatine group, then perhaps the dosage was not high enough to elicit other effects (positive or negative) within this population.

No significant differences were found in urine creatinine or creatinine clearance between the creatine and placebo groups either, although urine creatinine approached significance ($p=0.081$). A surplus of ingested creatine would be expected to increase renal elimination of creatinine (urine and creatinine clearance) in healthy individuals, or a buildup of creatinine in the blood or plasma (serum creatinine) in individuals with kidney dysfunction. Future studies performed at higher dosages could discover an optimal dosing protocol for this demographic and ascertain whether the dosage protocol in this study was insufficient to elicit results due to an absorption issue or muscle mass differences between young and older adults as has been suggested in previous research (Rawson et al., 2002). It is possible older adults require a higher standard

dosage of creatine to elicit physiological outcomes associated with creatine supplementation and determining the safety of supplementing at that level should be ascertained.

Another potential contributing factor to the lack of statistical significance found in some of the measures is participant sample size. Graphs of serum creatinine, urine creatinine, and creatinine clearance did show trends for expected differences, but were found not to be statistically significant. A larger participant pool with the same absolute differences would increase statistical power, although the pool size would likely have to be 200-300 participants to see significant differences as mentioned earlier.

While the need for additional long-term research in this population is needed, the present study indicates that for many of the outcome measures the largest differences from baseline were seen in the first 4-8 months. This suggests studies of 4-8 months would see the same or similar results with lower time commitment from both researchers and participants. More short-term studies (3-6 months) will be valuable to this pool of research and should show changes in physiological outcomes of participants, especially at higher dosages.

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Appendix A: Adverse Event Form

SUBJECT INITIALS: _____

RECRUITMENT ID #: _____

SERIOUS ADVERSE EVENT FORM

* This will be reported to the Research Ethics Board

Describe the adverse event: _____

(Record diagnosis where available or describe event in as few words as possible.)

1. Rate Intensity (severity): Please circle one

Mild

Moderate

Severe

Life threatening

2. Relationship to experimental procedure (supplement, exercise or other procedure):

Not related

Unlikely

Possible

Probable

Definite

Onset of Adverse Event (date/time): _____

Resolution of Adverse Event (date/time): _____

Action Taken: _____

Form completed by: _____

Date: _____

Signature of PI: _____

Date: _____

Appendix B: Liver Enzymes

Alanine aminotransferase (ALT) - A very high level of ALT is frequently seen with acute hepatitis. Moderate increases may be seen with chronic hepatitis. People with blocked bile ducts, cirrhosis, and liver cancer may have ALT concentrations that are only moderately elevated or close to normal.

Normal range 10-40 IU/L (5-45 U/L at local lab)

Normal ranges vary by age, gender, and lab.

Alkaline phosphatase (ALP) - ALP may be significantly increased with obstructed bile ducts, liver cancer, and also with bone disease.

ALP in liver disease

ALP results are usually evaluated along with other tests for liver disease. In some forms of liver disease, such as hepatitis, ALP is usually much less elevated than AST and ALT. When the bile ducts are blocked (usually by gallstones, scars from previous gallstones or surgery, or by cancers), ALP and bilirubin may be increased much more than AST or ALT. ALP may also be increased in liver cancer.

ALP in bone disease

In some bone diseases, such as Paget's disease, where bones become enlarged and deformed, or in certain cancers that spread to bone, ALP may be increased.

If a person is being successfully treated for Paget's disease, then ALP levels will decrease or return to normal over time. If someone with bone or liver cancer responds to treatment, ALP levels should decrease.

Moderately elevated ALP may result from other conditions, such as Hodgkin's lymphoma, congestive heart failure, ulcerative colitis, and certain bacterial infections.

Low levels of ALP may be seen temporarily after blood transfusions or heart bypass surgery. A deficiency in zinc may cause decreased levels. A rare genetic disorder of bone metabolism called hypophosphatasia can cause severe, protracted low levels of ALP. Malnutrition or protein deficiency as well as Wilson disease could also be possible causes for lowered ALP.

Normal Range 44-147 IU/L (30-110 U/L at local lab)

Normal ranges vary by age, gender, and lab.

Aspartate aminotransferase (AST) - A very high level of AST is frequently seen with acute hepatitis. AST may be normal to moderately increased with chronic hepatitis. In people with

blocked bile ducts, cirrhosis, and liver cancer, AST concentrations may be moderately increased or close to normal. When liver damage is due to alcohol, AST often increases much more than ALT (this is a pattern seen with few other liver diseases). AST is also increased after [heart attacks](#) and with muscle injury.

Normal Range 10-34 IU/L (10-35 U/L at local lab)* normal ranges may vary among labs.

Bilirubin - Bilirubin is increased in the blood when too much is being produced, less is being removed, due to bile duct obstructions, or to problems with bilirubin processing. It is not uncommon to see high bilirubin levels in newborns, typically 1 to 3 days old.

Normal Range 0.3-1.9 mg/dL; equivalent to 5.1-32.5 umol/L (2-22 umol/L at local lab)

Normal ranges vary by laboratory.

References:

Society for Biomedical Diabetes Research (SBDR) online; SI unit conversion calculator-
http://www.soc-bdr.org/rds/authors/unit_tables_conversions_and_genetic_dictionaries/conversion_in_si_units/index_en.html

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003473.htm>

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003470.htm>

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003472.htm>

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003479.htm>

All references retrieved June 25, 2014.

Appendix C: Liver Test Procedure Sheets

2076494322V6

ASTL

Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas[®]

* Indicates **cobas c** systems on which reagents can be used

Order information

Aspartate Aminotransferase acc. to IFCC

500 tests

Calibrator f.a.s. (12 x 3 mL)

Calibrator f.a.s. (12 x 3 mL, for USA)

Precinorm U plus (10 x 3 mL)

Precinorm U plus (10 x 3 mL, for USA)

Precipath U plus (10 x 3 mL)

Precipath U plus (10 x 3 mL, for USA)

Precinorm U (20 x 5 mL)

Precipath U (20 x 5 mL)

Diluent NaCl 9 % (50 mL)

Cat. No. **20764949** 322

Cat. No. **10759350** 190

Cat. No. **10759350** 360

Cat. No. **12149435** 122

Cat. No. **12149435** 160

Cat. No. **12149443** 122

Cat. No. **12149443** 160

Cat. No. **10171743** 122

Cat. No. **10171778** 122

Cat. No. **04489357** 190

System-ID 07 6494 9

Code 401

Code 401

Code 300

Code 300

Code 301

Code 301

Code 300

Code 301

System-ID 07 6869 3

Roche/Hitachi **cobas c** systems

cobas c 311 **cobas c** 501

English

System information

ASTL: ACN 687

SASTL: ACN 587 (STAT, reaction time: 7)

Intended use

In vitro test for the quantitative determination of aspartate aminotransferase (AST) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary^{1,2}

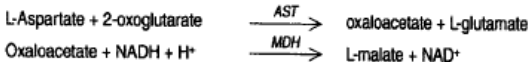
The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset.

In patients undergoing renal dialysis or those with vitamin B₆ deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of apoenzyme to holoenzyme. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the serum of patients with coronary and hepatobiliary disease.

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.^{3,4}

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD⁺.



The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 TRIS buffer: 264 mmol/L, pH 7.8 (37 °C); L-aspartate: 792 mmol/L; MDH (microorganism): ≥ 24 µkat/L; LDH (microorganisms): ≥ 48 µkat/L; albumin (bovine): 0.25 %; preservative

R2 NADH: ≥ 1.7 mmol/L; 2-oxoglutarate: 94 mmol/L; preservative

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

ASTL

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K₂-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay.

Stability: 24 hours at 15-25 °C⁵
7 days at 2-8 °C⁶

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

Distilled water

General laboratory equipment

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 test definition

Assay type	Rate A		
Reaction time / Assay points	10 / 12-31 (STAT 7 / 12-31)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (μkat/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	40 μL	51 μL	
R2	17 μL	20 μL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	9 μL	–	–
Decreased	9 μL	15 μL	135 μL
Increased	18 μL	–	–

cobas c 501 test definition

Assay type	Rate A		
Reaction time / Assay points	10 / 18-46 (STAT 7 / 18-46)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (μkat/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	40 μL	51 μL	
R2	17 μL	20 μL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	9 μL	–	–
Decreased	9 μL	15 μL	135 μL
Increased	18 μL	–	–

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ϵ .⁷

Quality control

For quality control, use control materials as listed in the "Order information" section.

Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factor: U/L \times 0.0167 = μkat/L

Limitations - interferences⁸

Criterion: Recovery within $\pm 10\%$ of initial value at an AST activity of 30 U/L (0.50 μkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 μmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 40 (approximate hemoglobin concentration: 25.6 μmol/L (40 mg/dL)).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic specimens may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{9,10}

Exception: Isoniazid causes artificially low AST results.

Cyanokit (Hydroxocobalamin) may cause interference with results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi

cobas c systems. Refer to the latest version of the Carry over evasion list found with the NaOH/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Measuring range

5-700 U/L (0.08-11.7 μkat/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.

Lower detection limit

5 U/L (0.08 μkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, $n = 21$).

Expected values¹¹

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation¹²):

Males: up to 40 U/L (up to 0.67 μkat/L)

Females: up to 32 U/L (up to 0.53 μkat/L)

Calculated values: A factor of 2.13 is used for the conversion from 25 °C to 37 °C.¹³

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol (within-run $n = 21$, total $n = 60$).

The following results were obtained:

Within-run	Mean U/L (μkat/L)	SD U/L (μkat/L)	CV %
Precinorm U	36.6 (0.61)	0.3 (0.01)	0.8
Precipath U	128 (2.14)	1 (0.02)	0.4
Human serum 1	126 (2.10)	1 (0.02)	0.4
Human serum 2	12.0 (0.20)	0.4 (0.01)	3.1
Total	Mean U/L (μkat/L)	SD U/L (μkat/L)	CV %
Precinorm U	36.7 (0.61)	0.5 (0.01)	1.3
Precipath U	130 (2.17)	1 (0.02)	0.8
Human serum 3	30.0 (0.50)	0.7 (0.01)	2.3
Human serum 4	121 (2.02)	2 (0.03)	1.9

ASTL

Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas®

Method comparison

AST values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c 501** analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 192

Passing/Bablok¹⁴

$y = 1.000x - 0.15 \text{ U/L}$

$r = 0.970$

Linear regression

$y = 0.991x + 1.22 \text{ U/L}$

$r = 0.999$

The sample activities were between 30.4 and 674 U/L (0.50 and 11.3 µkat/L).

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US Customer Technical Support 1-800-428-2336



ALT

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas®**Order information**• Indicates **cobas c** systems on which reagents can be used

Alanine Aminotransferase acc. to IFCC

500 tests

Calibrator f.a.s. (12 x 3 mL)

Calibrator f.a.s. (12 x 3 mL, for USA)

Precinorm U plus (10 x 3 mL)

Precinorm U plus (10 x 3 mL, for USA)

Precipath U plus (10 x 3 mL)

Precipath U plus (10 x 3 mL, for USA)

Precinorm U (20 x 5 mL)

Precipath U (20 x 5 mL)

Diluent NaCl 9 % (50 mL)

Cat. No. 20764957 322

Cat. No. 10759350 190

Cat. No. 10759350 360

Cat. No. 12149435 122

Cat. No. 12149435 160

Cat. No. 12149443 122

Cat. No. 12149443 160

Cat. No. 10171743 122

Cat. No. 10171778 122

Cat. No. 04489357 190

System-ID 07 6495 7

Code 401

Code 401

Code 300

Code 300

Code 301

Code 301

Code 300

Code 301

Code 301

System-ID 07 6869 3

Roche/Hitachi **cobas c** systems**cobas c** 311 **cobas c** 501**English****System information**

ALT: ACN 685

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary^{1,2}

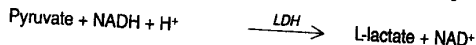
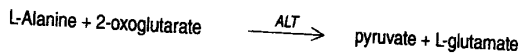
The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

In patients with vitamin B₆ deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.^{3,4} ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD⁺.



The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25 %; LDH (microorganisms): ≥ 45 µkat/L; stabilizers; preservative

R2 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

ALT

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis).

Plasma (free from hemolysis): Li-heparin and K₂-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum or plasma from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

Stability: 3 days at 15-25 °C^{6,7}

7 days at 2-8 °C^{6,7}

> 7 days at (-60)-(-80) °C⁷

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

Distilled water

General laboratory equipment

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

ALTL

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas®

Application for serum and plasma

cobas c 311 test definition

Assay type	Rate A
Reaction time / Assay points	10 / 12-31
Wavelength (sub/main)	700/340 nm
Reaction direction	Decrease
Units	U/L (µkat/L)
Reagent pipetting	
R1	59 µL
R2	17 µL
Sample volumes	
Normal	9 µL
Decreased	9 µL
Increased	18 µL

cobas c 501 test definition

Assay type	Rate A
Reaction time / Assay points	10 / 18-46
Wavelength (sub/main)	700/340 nm
Reaction direction	Decrease
Units	U/L (µkat/L)
Reagent pipetting	
R1	59 µL
R2	17 µL
Sample volumes	
Normal	9 µL
Decreased	9 µL
Increased	18 µL

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation, but without Pyp, using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ϵ .⁵

Quality control

For quality control, use control materials as listed in the "Order information" section.

Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factor: $U/L \times 0.0167 = \mu kat/L$

Limitations - interference⁶

Criterion: Recovery within $\pm 10\%$ of initial value at an ALT activity of 30 U/L (0.5 µkat/L).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L (200 mg/dL)).

cobas c systems

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid): No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic samples may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{9,10}

Exception: Calcium dobesilate and Isoniazid cause artificially low ALT results. Cyanokit (Hydroxocobalamin) may cause interference with results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi

cobas c systems. Refer to the latest version of the Carry over evasion list found with the NaOH/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Measuring range

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.

Lower detection limit

5 U/L (0.08 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, $n = 21$).

Expected values¹¹

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation¹²):

Males	up to 41 U/L (up to 0.68 µkat/L)
Females	up to 33 U/L (up to 0.55 µkat/L)

Calculated values: A factor of 1.85 is used for the conversion from 25 °C to 37 °C.¹³

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol (within-run $n = 21$, total $n = 60$).

The following results were obtained:

Within-run	Mean U/L (µkat/L)	SD U/L (µkat/L)	CV %
Precinorm U	39.5 (0.66)	0.3 (0.01)	0.6
Precipath U	120 (2.00)	1 (0.01)	0.4
Human serum 1	113 (1.89)	0.5 (0.01)	0.4
Human serum 2	7.2 (0.12)	0.7 (0.01)	9.3
Total	Mean U/L (µkat/L)	SD U/L (µkat/L)	CV %
Precinorm U	39.3 (0.66)	0.6 (0.01)	1.4
Precipath U	120 (2.00)	1 (0.02)	1.0
Human serum 3	24.0 (0.40)	0.6 (0.01)	2.6
Human serum 4	98.1 (1.64)	3.2 (0.05)	3.3

ALTL

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

Method comparison

ALT values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 198

Passing/Bablok¹⁴

$y = 1.000x - 0.29 \text{ U/L}$

$r = 0.924$

Linear regression

$y = 0.997x - 1.05 \text{ U/L}$

$r = 0.996$

The sample activities were between 4.6 and 383 U/L (0.08 and 6.4 $\mu\text{kat/L}$).

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14. Passing H, Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

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ALP2

Alkaline Phosphatase acc. to IFCC Gen.2

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* Indicates cobas c systems on which reagents can be used

Order information

Alkaline Phosphatase acc. to IFCC Gen.2

ALP2S 200 tests	Cat. No. 03333752 190	System-ID 07 6761 1
ALP2L 400 tests	Cat. No. 03333701 190	System-ID 07 6760 3
Calibrator f.a.s. (12 x 3 mL)	Cat. No. 10759350 190	Code 401
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. 10759350 360	Code 401
Precinorm U plus (10 x 3 mL)	Cat. No. 12149435 122	Code 300
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. 12149435 160	Code 300
Precipath U plus (10 x 3 mL)	Cat. No. 12149443 122	Code 301
Precipath U plus (10 x 3 mL, for USA)	Cat. No. 12149443 160	Code 301
Precinorm U (20 x 5 mL)	Cat. No. 10171743 122	Code 300
Precipath U (20 x 5 mL)	Cat. No. 10171778 122	Code 301
Diluent NaCl 9 % (50 mL)	Cat. No. 04489357 190	System-ID 07 6869 3

Roche/Hitachi cobas c systems

cobas c 311	cobas c 501
•	•
•	•

English

System information

ALP2S: ACN 158

ALP2L: ACN 683

Intended use

In vitro test for the quantitative determination of alkaline phosphatase in human serum and plasma on Roche/Hitachi cobas c systems.

Summary^{1,2,3,4,5,6}

Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from the germ cells. It occurs in osteoblasts, hepatocytes, leukocytes, the kidneys, spleen, placenta, prostate and the small intestine. The liver-bone-kidney type is particularly important.

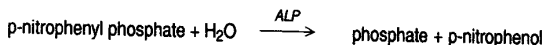
A rise in the alkaline phosphatase occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget's disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.

The assay method was first described by King and Armstrong, modified by Ohmori, Bessey, Lowry and Brock and later improved by Hausamen et al. In 1983 the International Federation of Clinical Chemistry (IFCC) recommended a standardized method for the determination of alkaline phosphatase using an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc. The assay described here is based on this recommendation, but was optimized for performance and stability. The assay was standardized against the IFCC reference formulation proposed above.

Test principle⁶

Colorimetric assay in accordance with a standardized method.

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.



The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 2-amino-2-methyl-1-propanol: 1.724 mol/L, pH 10.44 (30 °C);
magnesium acetate: 3.83 mmol/L; zinc sulfate: 0.766 mmol/L;
N-(2-hydroxyethyl)-ethylenediamine triacetic acid: 3.83 mmol/L

R2 p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.44 (30 °C); preservatives

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

2008-10, V 4 English

This kit contains components classified as follows according to the European directive 99/45/EEC:



R1 contains 2-Amino-2-methyl-1-propanol.

Irritant

R 36/38

Irritating to eyes and skin.

S 24/25

Avoid contact with skin and eyes.

Contact phone: all countries: +49-621-7590. USA: +1-800-428-2336

Reagent handling

Ready for use.

Storage and stability

ALP2S, ALP2L

Shelf life at 2-8 °C:

See expiration date on
cobas c pack label.

On-board in use and refrigerated on the analyzer:

8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:

See expiration date on
cobas c pack label.

On-board in use and refrigerated on the analyzer:

12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay.

Stability:⁷

7 days at 15-25 °C

7 days at 2-8 °C

2 months at (-15)-(-25) °C

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

Distilled water

General laboratory equipment

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

ALP2

Alkaline Phosphatase acc. to IFCC Gen.2

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 test definition

Assay type	Rate A	
Reaction time / Assay points	10 / 13-31	
Wavelength (sub/main)	480/450 nm	
Reaction direction	Increase	
Units	U/L (µkat/L)	
Reagent pipetting	Diluent (H ₂ O)	
R1	75 µL	25 µL
R2	17 µL	21 µL
Sample volumes	Sample	Sample dilution
		Sample Diluent (NaCl)
Normal	2.8 µL	-
Decreased	2.8 µL	20 µL 80 µL
Increased	5.6 µL	-

cobas c 501 test definition

Assay type	Rate A	
Reaction time / Assay points	10 / 19-48	
Wavelength (sub/main)	480/450 nm	
Reaction direction	Increase	
Units	U/L (µkat/L)	
Reagent pipetting	Diluent (H ₂ O)	
R1	75 µL	25 µL
R2	17 µL	21 µL
Sample volumes	Sample	Sample dilution
		Sample Diluent (NaCl)
Normal	2.8 µL	-
Decreased	2.8 µL	20 µL 80 µL
Increased	5.6 µL	-

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the proposed IFCC formulation⁶ using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, *c.*

Quality control

For quality control, use control materials as listed in the "Order information" section.

Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference⁸

Criterion: Recovery within ±10 % of initial value at an alkaline phosphatase activity of 100 U/L (1.67 µkat/L).

Icterus: No significant interference up to an I index of 35 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin

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concentration: 599 µmol/L (35 mg/dL) and approximate unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L (200 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{9,10}

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOH/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Measuring range

5-1200 U/L (0.084-20.0 µkat/L)

Determine samples having higher concentrations via the rerun function.

Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.

Lower detection limit

5 U/L (0.084 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Expected values

(measured at 37 °C)

Adults¹¹

Males (n = 221)	40-129 U/L	(0.67-2.15 µkat/L)
Females (n = 229)	35-104 U/L	(0.58-1.74 µkat/L)

Consensus values¹²

Males	40-130 U/L	(0.67-2.17 µkat/L)
Females	35-105 U/L	(0.58-1.75 µkat/L)

Children⁹

aged 1 day	<250 U/L	(<4.17 µkat/L)
aged 2-5 days	<231 U/L	(<3.84 µkat/L)
aged 6 days-6 months	<449 U/L	(<7.49 µkat/L)
aged 7 months-1 year	<462 U/L	(<7.69 µkat/L)
aged 1-3 years	<281 U/L	(<4.67 µkat/L)
aged 4-6 years	<269 U/L	(<4.48 µkat/L)
aged 7-12 years	<300 U/L	(<5.00 µkat/L)
aged 13-17 years (f)	<187 U/L	(<3.11 µkat/L)
aged 13-17 years (m)	<390 U/L	(<6.51 µkat/L)

a) Calculated from published reference ranges for the ALP opt. method (DGKC)¹³ using a factor of 0.417 derived from a method comparison.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

ALP2

Alkaline Phosphatase acc. to IFCC Gen.2

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run $n = 21$, total $n = 63$).

The following results were obtained:

Within-run	Mean	SD	CV
	U/L ($\mu\text{kat/L}$)	U/L ($\mu\text{kat/L}$)	%
Precinorm U	99.2 (1.65)	0.7 (0.01)	0.7
Precipath U	241 (4.02)	1.4 (0.02)	0.6
Human serum 1	54.6 (0.91)	0.5 (0.01)	0.9
Human serum 2	648 (10.8)	4.5 (0.08)	0.7
Total			
	Mean	SD	CV
	U/L ($\mu\text{kat/L}$)	U/L ($\mu\text{kat/L}$)	%
Precinorm U	92.8 (1.56)	2.2 (0.04)	2.4
Precipath U	224 (3.74)	3.8 (0.06)	1.7
Human serum 3	82.2 (1.37)	1.8 (0.03)	2.1
Human serum 4	1025 (17.1)	9.0 (0.15)	0.9

Method comparison

Alkaline phosphatase values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 203

Passing/Bablok¹⁴

$$y = 0.988x + 1.31 \text{ U/L}$$

$$r = 0.961$$

Linear regression

$$y = 0.991x + 0.80 \text{ U/L}$$

$$r = 0.997$$

The sample activities were between 50 and 1002 U/L (0.84 and 16.7 $\mu\text{kat/L}$).

References

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US Customer Technical Support 1-800-428-2336



BILTS

Total Bilirubin Special

cobas®

• Indicates cobas c systems on which reagents can be used

Order information

Total Bilirubin Special

250 tests	Cat. No. 03261638 190	System-ID 07 6721 2
Calibrator f.a.s. (12 x 3 mL)	Cat. No. 10759350 190	Code 401
Precinorm U plus (10 x 3 mL)	Cat. No. 12149435 122	Code 300
Precipath U plus (10 x 3 mL)	Cat. No. 12149443 122	Code 301
Precinorm U (20 x 5 mL)	Cat. No. 10171743 122	Code 300
Precipath U (20 x 5 mL)	Cat. No. 10171778 122	Code 301
Precibil (4 x 2 mL)	Cat. No. 10158046 122	Code 306
PreciControl ClinChem Multi 1 (20 x 5 mL)	Cat. No. 05117003 190	Code 391
PreciControl ClinChem Multi 2 (20 x 5 mL)	Cat. No. 05117216 190	Code 392
Diluent NaCl 9 % (50 mL)	Cat. No. 04489357 190	System-ID 07 6869 3

Roche/Hitachi **cobas c** systems

cobas c 311 **cobas c** 501/502

English

System information

For **cobas c** 311/501 analyzers:

BILTS: ACN 18

SBILS: ACN 62 (STAT, reaction time: 5)

For **cobas c** 502 analyzer:

BILTS: ACN 8018

SBILS: ACN 8062 (STAT, reaction time: 5)

Intended use

In vitro test for the quantitative determination of total bilirubin in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary¹

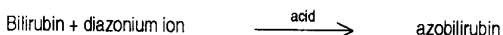
Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract.

Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Test principle

Diazo method (special).²

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a diazonium ion in a strongly acidic medium (pH 1-2).



The intensity of the color of the azobilirubin produced is proportional to the total bilirubin concentration and can be measured photometrically.

Reagents - working solutions

R1 Sulfamic acid (H₃NO₃S): 110 mmol/L; sodium acetate buffer (C₂H₃NaO₂): 85 mmol/L; surfactant; solubilizer

R2 Diazonium ion: 3 mmol/L; HCl: 100 mmol/L

R1 is in position B, and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

BILTS

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

5 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Li-heparin and K₂-EDTA plasma

Underfilled lithium heparin sample tubes may cause elevated results.

Verify all neonatal T-Bilirubin results in heparin samples in the therapeutic decision range.

Do not use cord blood samples.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:^{a,3} 1 day at 15-25 °C
7 days at 2-8 °C
6 months at (-15)-(-25) °C

a) If care is taken to prevent exposure to light

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

BILTS

Total Bilirubin Special

cobas®

Application for serum and plasma

cobas c 311 test definition

Assay type	2 Point End		
Reaction time / Assay points	10 / 6-22 (STAT 5 / 6-22)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	120 µL	–	
R2	31 µL	–	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 501/502 test definition

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-31 (STAT 5 / 10-31)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	120 µL	–	
R2	31 µL	–	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration - after 7 days on board - after reagent lot change - as required following quality control procedures

Traceability: The method was standardized against the Doumas method.⁴

Quality Control

For quality control, use control materials as listed in the "Order information" section.
In addition, other suitable control material can be used.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: µmol/L x 0.0585 = mg/dL
mg/dL x 10 = mg/L
mg/dL x 17.1 = µmol/L

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a total bilirubin concentration of 17 µmol/L (1.0 mg/dL).

Hemolysis:⁵ No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 µmol/L (500 mg/dL)).

Lipemia (Intralipid):⁵ No significant interference up to an L index of 600. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{6,7}

Indican: No significant interference from indican up to levels of 10 mg/dL or 100 mg/L.

Exception: Ascorbic acid at 30 mg/dL causes artificially low total bilirubin results. Cyanokit (Hydroxocobalamin) may cause interference with results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Results from certain multiple myeloma patients may show a positive bias in recovery. Not all multiple myeloma patients show the bias and the severity of the bias may vary between patients.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both D-bilirubin and total bilirubin values.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCin1 + 2/SCCS Method Sheets. For further instructions refer to the operator's manual.

cobas c 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

1.7-600 µmol/L (0.1-35.1 mg/dL)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit of the test

1.7 µmol/L (0.1 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Adults ⁸	up to 21 µmol/L	(up to 1.2 mg/dL)
Children with age ≥ 1 month ⁸	up to 17 µmol/L	(up to 1.0 mg/dL)
Reference range study with 500 well-characterized human serum samples ⁹ :		
Males	up to 24 µmol/L	(up to 1.4 mg/dL)
Females	up to 15 µmol/L	(up to 0.9 mg/dL)

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term¹⁰

Age of newborn:

24 hours	≥ 137* µmol/L	(≥ 8.0* mg/dL)
48 hours	≥ 222* µmol/L	(≥ 13.0* mg/dL)
84 hours	≥ 290* µmol/L	(≥ 17.0* mg/dL)

* 95th percentile

Levels > 95th percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

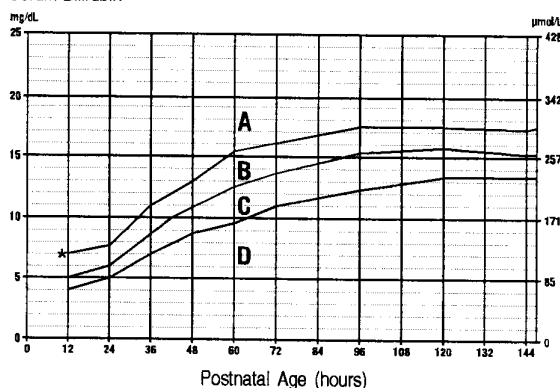
BILTS

Total Bilirubin Special

cobas®

Nomogramm for designation of risk in 2840 well newborns¹⁰

Serum Bilirubin



*95th percentile

A High risk zone

C Low intermediate risk zone

B High intermediate risk zone

D Low risk zone

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability* (n = 21) and intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability*	Mean	SD	CV
	μmol/L (mg/dL)	μmol/L (mg/dL)	%
Precinorm U	16.7 (0.977)	0.4 (0.023)	2.5
Precipath U	84.8 (4.96)	1.0 (0.06)	1.2
Human serum 1	55.4 (3.24)	0.5 (0.03)	0.9
Human serum 2	13.0 (0.761)	0.3 (0.018)	2.1
Intermediate precision**	Mean	SD	CV
	μmol/L (mg/dL)	μmol/L (mg/dL)	%
Precinorm U	20.4 (1.19)	0.8 (0.05)	3.9
Precipath U	80.7 (4.72)	1.6 (0.10)	2.0
Human serum 3	11.9 (0.696)	0.6 (0.035)	4.6
Human serum 4	57.3 (3.35)	1.8 (1.05)	3.1

* repeatability = within-run precision

** intermediate precision = total precision / between run / between day precision

Method comparison

Total bilirubin values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared to those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 261

Passing/Bablok¹¹

y = 1.002x - 2.21 μmol/L

r = 0.976

Linear regression

y = 1.006x - 2.37 μmol/L

r = 1.000

The sample concentrations were between 1.80 and 568 μmol/L (0.105 and 33.2 mg/dL).

References

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10. Subcommittee on Hyperbilirubinemia. Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation. Pediatrics 2004;114:297-316.
11. Passing H, Bablok W, Bender R, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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Appendix D: Kidney Measures Information Sheet

Serum Creatinine - The creatinine blood test is used along with a [BUN \(blood urea nitrogen\) test](#) to assess kidney function. Both are frequently ordered as part of a [basic](#) or [comprehensive metabolic panel](#) (BMP or CMP), groups of tests that are performed to evaluate the function of the body's major organs. Serum creatinine measurements (along with your age, weight, and gender) also are used to calculate the [estimated glomerular filtration rate \(eGFR\)](#), which is used as a screening test to look for evidence of kidney damage.

Increased creatinine levels in the blood suggest diseases or conditions that affect kidney function. Creatinine blood levels can also increase temporarily as a result of muscle injury and are generally slightly lower during [pregnancy](#). Low blood levels of creatinine are not common, but they are also not usually a cause for concern. They can be seen with conditions that result in decreased muscle mass.

Normal Range = 0.7-1.3 mg/dL (men) and 0.6-1.1 mg/dL (women); equivalent to 61.9-114.9 umol/L and 53.0-97.2 umol/L respectively. Normal range for local lab = 45-110 umol/L

Normal ranges are generally higher in men vs. women and vary among labs.

Urine Creatinine - Levels of 24-hour urine creatinine are evaluated with blood levels as part of a [creatinine clearance test](#).

A combination of blood and urine creatinine levels may be used to calculate a creatinine clearance. This test measures how effectively the kidneys are filtering small molecules like creatinine out of the blood.

Urine creatinine may also be used with a variety of other urine tests as a correction factor. Since it is produced and removed at a relatively constant rate, the amount of urine creatinine can be compared to the amount of another substance being measured. This stable excretion rate is useful when evaluating both 24-hour urine samples and random urine samples. Examples of this are when creatinine is measured with protein to calculate a urine protein/creatinine ratio (UP/CR) and when it is measured with microalbumin to calculate a microalbumin/creatinine ratio.

The microalbumin/creatinine ratio is calculated to help determine how much albumin is escaping from the kidneys into the urine. People who have consistently detectable amounts of albumin in their urine (microalbuminuria) have an increased risk of developing progressive kidney failure and cardiovascular disease in the future.

Normal Range = 14-26 mg/kg (men) and 11-20 mg/kg (women); LOCAL LAB RANGE???

Normal ranges are generally higher in men vs. women and vary by lab.

Creatinine Clearance - A creatinine clearance test is used to help evaluate the rate and efficiency of kidney filtration. It is used to help detect and diagnose kidney dysfunction and/or the presence of decreased blood flow to the kidneys. The creatinine clearance test may be ordered whenever a doctor wants to evaluate the filtration ability of the kidneys.

Any disease or condition that affects the [glomeruli](#) can decrease the kidneys' ability to clear creatinine and other wastes out of the blood. When this occurs, the [blood creatinine level](#) will be increased and the creatinine clearance will be decreased because not as much creatinine is able to be excreted in the urine.

A decreased creatinine clearance rate may also occur when there is decreased blood flow to the kidneys as may occur with [congestive heart failure](#), [obstruction within the kidney](#), or [acute](#) or [chronic](#) kidney failure. The less effective the kidney filtration, the greater the decrease in clearance. Increased creatinine clearance rates may occasionally be seen during [pregnancy](#), exercise, and with diets high in meat, although this test is not typically used to monitor these conditions.

Normal Range = 97-137 ml/min (men) and 88-128 ml/min (women). Normal range for local lab = 74-125 ml/min

Normal ranges are generally higher in men vs. women and vary by lab.

References:

Lab Tests Online - <http://labtestsonline.org/understanding/analytes/creatinine/tab/test/>

Lab Tests Online - <http://labtestsonline.org/understanding/analytes/creatinine-clearance/tab/test/>

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003610.htm>

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003611.htm>

Medline Plus online - <http://www.nlm.nih.gov/medlineplus/ency/article/003475.htm>

Society for Biomedical Diabetes Research (SBD R) online; SI unit conversion calculator-
[http://www.soc-](http://www.soc-bdr.org/rds/authors/unit_tables_conversions_and_genetic_dictionaries/conversion_in_si_units/index_en.html)

[bdr.org/rds/authors/unit_tables_conversions_and_genetic_dictionaries/conversion_in_si_units/index_en.html](http://www.soc-bdr.org/rds/authors/unit_tables_conversions_and_genetic_dictionaries/conversion_in_si_units/index_en.html)

All references retrieved June 25, 2014.

Appendix E: Kidney Testing Reference Sheets

03263991180V10

CREP2

Creatinine plus ver.2

cobas®

* Indicates **cobas c** systems on which reagents can be used

Order information

Creatinine plus ver.2

250 tests	Cat. No. 03263991 190	System-ID 07 6612 7
Calibrator f.a.s. (12 x 3 mL)	Cat. No. 10759350 190	Code 401
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. 10759350 360	Code 401
Precinorm U plus (10 x 3 mL)	Cat. No. 12149435 122	Code 300
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. 12149435 160	Code 300
Precipath U plus (10 x 3 mL)	Cat. No. 12149443 122	Code 301
Precipath U plus (10 x 3 mL, for USA)	Cat. No. 12149443 160	Code 301
Precinorm U (20 x 5 mL)	Cat. No. 10171743 122	Code 300
Precipath U (20 x 5 mL)	Cat. No. 10171778 122	Code 301
Precinorm PUC (4 x 3 mL)	Cat. No. 03121313 122	Code 240
Precipath PUC (4 x 3 mL)	Cat. No. 03121291 122	Code 241
PreciControl ClinChem Multi 1 (20 x 5 mL)	Cat. No. 05117003 190	Code 391
PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Cat. No. 05947626 160	Code 391
PreciControl ClinChem Multi 2 (20 x 5 mL)	Cat. No. 05117216 190	Code 392
PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Cat. No. 05947774 160	Code 392
Diluent NaCl 9 % (50 mL)	Cat. No. 04489357 190	System-ID 07 6869 3

Roche/Hitachi **cobas c** systems

cobas c 311 **cobas c** 501/502

English

System information

For **cobas c** 311/501 analyzers:

CREA2: ACN 452 (serum, plasma, urine)

For **cobas c** 502 analyzer:

CREA2: ACN 8452 (serum, plasma)

CRE2U: ACN 8152 (urine)

Intended use

In vitro assay for the quantitative determination of creatinine in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary^{1,2,3,4,5}

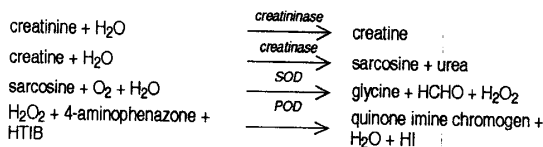
Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m² for three months or more, regardless of cause. The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockcroft and Gault and that based on the results of the MDRD trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Bedside Schwartz formula should be used.^{6,7,8,9}

In addition to the diagnosis and treatment of renal disease, the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.

Test principle

This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. Catalyzed by peroxidase the liberated hydrogen peroxide reacts with 4-aminophenazone and HTIB^a to form a quinone imine chromogen. The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration in the reaction mixture.



Creatine of the sample is destroyed by creatinase, SOD and catalase during incubation in R1.

a) 2,4,6-triiodo-3-hydroxybenzoic acid

Reagents - working solutions

R1 TAPS buffer (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid): 30 mmol/L, pH 8.1; creatinase (microorganisms): ≥ 332 μkat/L; sarcosine oxidase (microorganisms): ≥ 132 μkat/L; ascorbate oxidase (microorganisms): ≥ 33 μkat/L; catalase (microorganisms): ≥ 1.67 μkat/L; HTIB: 1.2 g/L; detergents; preservative

R3 TAPS buffer: 50 mmol/L, pH 8.0; creatininase (microorganisms): ≥ 498 μkat/L; peroxidase (horseradish): ≥ 16.6 μkat/L; 4-aminophenazone: 0.5 g/L; potassium hexacyanoferrate (II): 60 mg/L; detergent; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

CREP2

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

8 weeks

CREP2

Creatinine plus ver.2

Diluent NaCl 9 %

Shelf life at 2-8 °C:

See expiration date on
cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K₂-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine.

Collect urine without using additives. If urine must be collected with a preservative for other analytes, only hydrochloric acid (14 to 47 mmol/L urine, e.g. 5 mL 10 % HCl or 5 mL 30 % HCl per liter urine) or boric acid (81 mmol/L, e.g. 5 g per liter urine) may be used.

Stability in *serum/plasma*:¹⁰
7 days at 15-25 °C
7 days at 2-8 °C
3 months at (-15)-(-25) °C

Stability in *urine* (without preservative):¹⁰
2 days at 15-25 °C
6 days at 2-8 °C
6 months at (-15)-(-25) °C

Stability in *urine* (with preservative):¹⁰
3 days at 15-25 °C
8 days at 2-8 °C
3 weeks at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

General laboratory equipment

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 501/502 test definition

Assay type 2 Point End
Reaction time / Assay points 10 / 37-70
Wavelength (sub/main) 700/546 nm
Reaction direction Increase
Units μmol/L (mg/dL, mmol/L)

Reagent pipetting Diluent (H₂O)
R1 77 μL -
R3 38 μL -

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 μL	-	-
Decreased	5 μL	15 μL	135 μL
Increased	4 μL	-	-

cobas c 311 test definition

Assay type 2 Point End
Reaction time / Assay points 10 / 25-57
Wavelength (sub/main) 700/546 nm
Reaction direction Increase
Units μmol/L (mg/dL, mmol/L)

Reagent pipetting Diluent (H₂O)
R1 77 μL -
R3 38 μL -

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 μL	-	-
Decreased	5 μL	15 μL	135 μL
Increased	4 μL	-	-

Application for urine

cobas c 501/502 test definition

Assay type 2 Point End
Reaction time / Assay points 10 / 37-70
Wavelength (sub/main) 700/546 nm
Reaction direction Increase
Units μmol/L (mg/dL, mmol/L)

Reagent pipetting Diluent (H₂O)
R1 77 μL -
R3 38 μL -

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	5 μL	3 μL	147 μL
Decreased	2 μL	3 μL	147 μL
Increased	10 μL	3 μL	147 μL

cobas c 311 test definition

Assay type 2 Point End
Reaction time / Assay points 10 / 25-57
Wavelength (sub/main) 700/546 nm
Reaction direction Increase
Units μmol/L (mg/dL, mmol/L)

Reagent pipetting Diluent (H₂O)
R1 77 μL -
R3 38 μL -

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	5 μL	3 μL	147 μL
Decreased	2 μL	3 μL	147 μL
Increased	10 μL	3 μL	147 μL

Calibration

Calibrators S1: H₂O
S2: C.f.a.s.
Calibration mode Linear
Calibration frequency Blank calibration
- after 4 weeks during shelf life
2-point calibration
- after reagent lot change
- as required following quality control procedures

Traceability: This method has been standardized against ID/MS.

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CREP2

Creatinine plus ver.2

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Quality control

Serum/plasma

For quality control use undiluted Precinorm U, Precipath U, PreciControl ClinChem Multi 1 and PreciControl ClinChem Multi 2 as listed above. Other suitable control material can be used in addition.

Urine

For quality control use Precinorm PUC and Precipath PUC as listed above. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits. Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors: $\mu\text{mol/L} \times 0.0113 = \text{mg/dL}$
 $\mu\text{mol/L} \times 0.001 = \text{mmol/L}$

Limitations - interference

Criterion: Recovery within $\pm 10\%$ of initial values at creatinine concentrations of 80 $\mu\text{mol/L}$ (0.9 mg/dL) in serum and 2500 $\mu\text{mol/L}$ (28.3 mg/dL) in urine.

Serum/plasma

Icterus:¹² No significant interference up to an I index of 15 for conjugated bilirubin and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 257 $\mu\text{mol/L}$ or 15 mg/dL; approximate unconjugated bilirubin concentration: 342 $\mu\text{mol/L}$ or 20 mg/dL).

Hemolysis:¹² No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 $\mu\text{mol/L}$ or 800 mg/dL).

Lipemia (Intralipid):¹² No significant interference up to an L index of 2000. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ascorbic acid: < 1.70 mmol/L or < 300 mg/L does not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{13,14}

Exceptions: Rifampicin, Levodopa and Calcium dobesilate (e.g. Dexium) cause artificially low creatinine results.

N-ethylglycine at therapeutic concentrations and DL-proline at concentrations $\geq 1 \text{ mmol/L}$ ($\geq 115 \text{ mg/L}$) give falsely high results.

No significant interference up to a creatine level of 4 mmol/L (524 mg/L).

Hemolyzed samples from neonates, infants or adults with HbF values $\geq 600 \text{ mg/dL}$ interfere with the test.¹⁵

2-Phenyl-1,3-indandion (Phenindion) at therapeutic concentrations interferes with the assay.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Estimation of the glomerular filtration rate (GFR) on the basis of the Schwartz formula can lead to an overestimation.¹⁶

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Urine

Icterus: No significant interference up to an approximate conjugated bilirubin concentration of 1197 $\mu\text{mol/L}$ (70 mg/dL).

Hemolysis: No significant interference up to an approximate hemoglobin concentration of 621 $\mu\text{mol/L}$ (1000 mg/dL).

Ascorbic acid < 22.7 mmol/L (< 4000 mg/L), glucose < 120 mmol/L (< 2162 mg/dL) and urobilinogen < 676 $\mu\text{mol/L}$ (< 40 mg/dL) do not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.¹⁴

Exceptions: Calcium dobesilate (e.g. Dexium), Levodopa and α -methyl dopa cause artificially low creatinine results.

High homogentisic acid concentrations in urine samples lead to false results.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the Carry-over evasion list can be found with the NaOH/SMS/Multiclear/SCCS or the NaOH/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator's manual.

cobas c 502 analyzer: All special wash programming necessary for avoiding carry-over is available via cobas link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Serum/plasma

5-2700 $\mu\text{mol/L}$ (0.06-30.5 mg/dL)

Determine samples having higher concentrations via the rerun function.

Dilution of samples via the rerun function is a 1:4 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 4.

Urine

100-54000 $\mu\text{mol/L}$ (1.1-610 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Lower detection limit of the test

Serum/plasma

5 $\mu\text{mol/L}$ (0.06 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, $n = 21$).

Urine

100 $\mu\text{mol/L}$ (1.1 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, $n = 21$).

Expected values

Serum/plasma

Adults¹⁷

Females	45-84 $\mu\text{mol/L}$	(0.51-0.95 mg/dL)
Males	59-104 $\mu\text{mol/L}$	(0.67-1.17 mg/dL)

Children¹⁸

Neonates (premature)	29-87 $\mu\text{mol/L}$	(0.33-0.98 mg/dL)
Neonates (full term)	27-77 $\mu\text{mol/L}$	(0.31-0.88 mg/dL)
2-12 m	14-34 $\mu\text{mol/L}$	(0.16-0.39 mg/dL)
1- < 3 y	15-31 $\mu\text{mol/L}$	(0.18-0.35 mg/dL)
3- < 5 y	23-37 $\mu\text{mol/L}$	(0.26-0.42 mg/dL)
5- < 7 y	25-42 $\mu\text{mol/L}$	(0.29-0.47 mg/dL)
7- < 9 y	30-47 $\mu\text{mol/L}$	(0.34-0.53 mg/dL)
9- < 11 y	29-56 $\mu\text{mol/L}$	(0.33-0.64 mg/dL)
11- < 13 y	39-60 $\mu\text{mol/L}$	(0.44-0.68 mg/dL)
13- < 15 y	40-68 $\mu\text{mol/L}$	(0.46-0.77 mg/dL)

Urine

1st morning urine¹⁷

Females	2.55-20.0 mmol/L	(29-226 mg/dL)
Males	3.54-24.6 mmol/L	(40-278 mg/dL)

24-hour urine¹⁹

Females	6-13 mmol/24 h	(720-1510 mg/24 h)
Males	9-19 mmol/24 h	(980-2200 mg/24 h)

Creatinine clearance¹⁹

	66-143 mL/min
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Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. Roche has not evaluated reference ranges in a pediatric population.

CREP2

Creatinine plus ver.2

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. **Serum/plasma:** Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days).

Urine: Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum/plasma

Repeatability*	Mean μmol/L (mg/dL)	SD μmol/L (mg/dL)	CV %
Precinorm U	96.1 (1.08)	0.9 (0.01)	0.9
Precipath U	341 (3.85)	2 (0.02)	0.6
Human serum 1	191 (2.16)	2 (0.02)	1.1
Human serum 2	398 (4.50)	4 (0.05)	1.0

Intermediate precision**	Mean μmol/L (mg/dL)	SD μmol/L (mg/dL)	CV %
Precinorm U	94.9 (1.07)	1.4 (0.02)	1.4
Precipath U	338 (3.82)	4 (0.05)	1.1
Human serum 3	190 (2.15)	2 (0.02)	1.1
Human serum 4	395 (4.46)	5 (0.06)	1.2

Urine

Repeatability*	Mean μmol/L (mg/dL)	SD μmol/L (mg/dL)	CV %
Control Level 1	7280 (82.3)	92 (1.0)	1.3
Control Level 2	14031 (159)	179 (2)	1.3
Human urine 1	17289 (195)	237 (3)	1.4
Human urine 2	7035 (79.5)	68 (0.8)	1.0

Intermediate precision**	Mean μmol/L (mg/dL)	SD μmol/L (mg/dL)	CV %
Control Level 1	7219 (81.6)	112 (1.3)	1.5
Control Level 2	14018 (158)	212 (2)	1.5
Human urine 3	17326 (196)	244 (3)	1.4
Human urine 4	7008 (79.2)	104 (1.2)	1.5

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

Creatinine values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c 501** analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Serum/plasma

Sample size (n) = 63

Passing/Bablok²⁰

$$y = 1.002x - 0.434 \mu\text{mol/L}$$

$$r = 0.978$$

Linear regression

$$y = 0.991x + 2.94 \mu\text{mol/L}$$

$$r = 1.000$$

The sample concentrations were between 48.9 and 1891 μmol/L (0.553 and 21.4 mg/dL).

Urine

Sample size (n) = 75

Passing/Bablok²⁰

$$y = 0.985x + 21.3 \mu\text{mol/L}$$

$$r = 0.990$$

Linear regression

$$y = 0.977x + 80.0 \mu\text{mol/L}$$

$$r = 1.000$$

The sample concentrations were between 438 and 52577 μmol/L (4.95 and 594 mg/dL).

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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